COPULATION BEHAVIOUR, PATERNITY AND GENETIC RELATEDNESS IN COMMON MURRES (Uria aalge)

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Copulation Behaviour, Paternity and Genetic Relatedness in Common Murres (*Uria aalge*)

by

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Abstract

In order to examine the relationship between extra-pair copulation (EPC) behaviour and extra-pair paternity (EPP) in Common Murres (*Uria aalge*), this study combined four breeding seasons of field observations on the copulation behaviour of a marked subpopulation of murres with genetic analyses of EPP in chicks. The genetic relatedness of individuals between and within two Newfoundland seabird colonies was also examined in order to determine 1) if genetic relatedness among individuals within a ledge affected their EPC behaviour, and 2) the degree of micro- and macro-geographic population differentiation in these murres.

Behavioural observations indicated that few extra-pair copulations (EPCs) attempted by males were accepted by females. Contrary to previous studies, I found no evidence that male murres could force cloacal contact with females that resisted EPCs. A disproportionate number of females that accepted EPCs were in unstable pair bonds that were terminated during the study (i.e., the pairs divorced). Divorced female's acceptance of EPCs occurred both prior to and after divorce in most cases, suggesting that some EPCs were used by these females as a means of mate sampling. Male EPC behaviour was unrelated to pair bond stability.

Paternity analyses were conducted using four microsatellite loci on 30 families sampled from 1996-1999. Only three cases of EPP were detected, all in 1998, indicating an overall EPP rate of approximately 10%. Two cases of EPP involved pairs which

divorced in the year following the production of an extra-pair chick. In contrast to most female murres who accepted pair copulations (PCs) following EPCs, the two females with an EP chick that were observed during pre-laying refused all PC attempts by their mates. This suggests that females may modify their acceptance of PCs in order to ensure that EPCs result in extra-pair fertilization (EPF). Overall, both copulation behaviour and paternity outcome was largely controlled by females. The clustering of all EPP cases in one year may indicate significant among-year variation in EPP rates for long-lived species such as Common Murres.

Relatedness analyses indicated that two ledges contained murres that were related at the approximate level of first cousins, but other ledges/areas showed low average relatedness coefficients. The genetic markers used were able to differentiate known first-degree relatives and unrelated dyads on average, although there was high variability among pairwise relatedness estimates. Social mates, as well as extra-pair mates, were generally unrelated.

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Chapter 1
Introduction and Overview

The integration of molecular techniques with field studies of social behaviour has revolutionized our understanding of mating systems, the social structure of groups, and how social behaviour has evolved (and is evolving) in many taxa (Hughes 1998). The demise of simplistic notions such as monogamy, particularly in birds, for example, is one result of this molecular revolution. Traditionally, approximately 90% of all avian species were believed to be socially and sexually monogamous (Lack 1968). It has since been realized that for many birds, patterns of genetic and social monogamy differ drastically: partners within some socially monogamous species participate in extra-pair copulations (EPCs) that result in extra-pair fertilizations (EPFs; e.g., Swallows, *Hirunda rustica*, Primmer et al. 1995; Short-tailed Shearwater, *Puffinus tenuirostris*, Austin and Parkin 1996; Common Gulls, *Larus canus*, Bukacinska et al. 1998; Great Tits, *Parus major*, Lubjuhn 1999). Just as interestingly, molecular paternity analyses have determined that, in other species, genetic and social monogamy co-exist, in that there is no evidence of extra-pair paternity (EPP) despite the occurrence of EPCs (e.g., Northern Fulmars, *Fulmaris glacialis*, Hunter et al. 1992; Wilson's Storm Petrels, *Oceanites oceanicus*, Quillfeldt et al. 2001; Cory's Shearwater, *Calonectris diomedea*, Rabouam 2000).

Factors which determine whether EPCs are performed (*How many and which males attempt EPCs?*), whether they are successful (*How many and which females solicit and/or accept EPCs?*), and whether they result in a fertilization (*What proportion of successful EPCs lead to EPFs?*) are not yet well understood. In my opinion, this is partly

due to the rash of molecular studies which have not encompassed significant behavioural observations of individuals (e.g., Graves et al. 1992; Austin and Parkin 1996; Bukacinska et al. 1998; Taylor et al. 2000; for exceptions, see Hunter et al. 1992; Swatschek et al. 1994; Schwartz et al. 1999). Even in studies which have examined EPP rates and EPC behaviour concurrently, most are unable to link individual behaviour to paternity results. Within the literature on mating systems, however, there appears to be a recent shift to the recognition that behavioural observations (preferably long-term) and paternity analyses are both required in order for us to answer fundamental questions such as "*Why* do individuals perform EPCs?", "*How* and *when* does EPP come about?", and "*What* are the relationships between EPC behaviour and other behavioural or demographic factors?" (e.g., Lubjuhn et al. 1999; Buchanan and Catchpole 2000; Green et al. 2000). In part, the question "*Who* performs EPCs?" lies at the heart of answers which are so critical to our understanding of avian mating behaviour, in general, and EPC behaviour, in particular.

The contribution of molecular genetic techniques to current knowledge of population structure of mammals, fish, insects, and birds has also been significant (Hughes 1998). Studies have investigated both the social and genetic structure within and among populations, and have generated results with relevance to breeding dispersal of individuals, philopatry, inbreeding, gene flow among populations, and taxonomy/phylogeny (for birds, see Avise 1996; for social insects, see Ross 2001; for mammal examples, see Palsbøll 1999). Apart from their obvious theoretical relevance (for example, to the evolution of kin selection; Hamilton 1964), such analyses can have important implications for the preservation of genetic variability within these populations,

and, hence, may have direct conservation consequences as well (Sugg et al. 1996; Beaumont and Bruford 1999).

Various molecular techniques, each with their unique strengths and weaknesses, have been employed to analyze the genetic relationships within and among social groups (reviews in Schlötterer and Pemberton 1994; Fleischer 1996; Parker et al. 1998). The most common manner of determining paternity, in particular, has been with variable number of tandem repeat (VNTR) markers in two forms: 1) multi-locus DNA fingerprinting and 2) single-locus microsatellites. DNA fingerprinting typically involves the use of minisatellites, tandem repeats of DNA consisting of motifs approximately 9-65 base pairs, which are used to screen several hypervariable loci simultaneously to produce individual-specific patterns of DNA (analogous to individual fingerprints; Ellegren 1992). While DNA fingerprinting has been used successfully in many studies (e.g., Swatschek et al. 1994; Birkhead et al. 2001), the technique has several disadvantages when compared to microsatellite techniques (Fleischer 1996). For example, with DNA fingerprinting, putative parents and offspring should be run on the same gels (a problem if samples of potential fathers and chicks, for example, are not obtained contemporaneously), and greater amounts of DNA per individual may be required to complete analyses. As well, unlike microsatellites, specific loci and alleles usually cannot be determined (Fleischer 1996).

Microsatellites, on the other hand, involve amplification of individual genetic loci and permit the heterozygosity and number of alleles to be estimated for each locus

(Paetkau and Strobeck 1994; Jarne and Lagoda 1996). Because microsatellites, randomly dispersed segments of DNA consisting of tandem repeats of 1-5 nucleotides, are often hypervariable and are inherited in a Mendelian fashion, they are useful as polymorphic markers that can identify both individuals and the genetic relationships among individuals (Ellegren 1992; Queller et al. 1993; Jarne and Lagoda 1996). One of the largest drawbacks to the use of microsatellites is that the development of the primers that amplify the microsatellites during PCR is often difficult and time-consuming, and, as they are created from the DNA of a particular study species, these primers often will only cross-anneal with other closely-related species (Fleischer 1996). Indeed, even congeneric species may exhibit significant differences in the heterozygosity observed at any given locus, or can differ with respect to whether a null allele is present at a locus (Ibarguchi et al. 2000). Such species-differences may reflect true species differences at these loci or may be artefacts of the primer design (Paetkau and Strobeck 1995). In addition, the power of any analyses using microsatellites generally increases as the number of loci and their heterozygosities increase (Blouin et al. 1996). Thus, with a limited set of loci, the power to examine paternity and the relatedness of individuals can be lower than desired.

Microsatellites designed in Thick-billed (*Uria lomvia*) and Common Murres (*U. aalge*; Ibarguchi et al. 2000) were used to investigate the incidence of EPP (Chapter 3) and genetic relatedness (Chapter 4) among Common Murres, in the context of a detailed analysis of mating behaviour in a group of marked individuals observed from 1997-2000 (Chapter 2). Common Murres, of the Family Alcidae, are mainly pelagic, migratory seabirds that come to land only during the breeding season, during which they breed

colonially on cliff ledges in the Northern Hemisphere (Tuck 1960; Harris and Birkhead 1985; Gaston and Jones 1998). They are widely distributed throughout the Hemisphere, with five races, based on morphological variation, having been described in Atlantic colonies (Gaston and Jones 1998). Murre pairs produce, at most, one chick per year, typically in the same territory or nest site, and with the same mate from year-to-year (Harris and Birkhead 1985; Gaston and Jones 1998). Both the male and female parents incubate eggs, brood chicks, and feed chicks fairly equally (Wanless and Harris 1986; S.I Wilhelm and A.E. Storey, unpublished data), but when the chick leaves the colony at approximately 3 weeks of age, the male parent accompanies it to sea and is believed to remain with the chick for two months (Gaston and Jones 1998). Typically, breeding is delayed in murres until about 5 years of age, when many individuals return to their natal colony to breed (Hudson 1985; Halley et al. 1995). Divorce rates are low (~12% in some UK colonies, Harris, pers. comm. cited in Black 1996; <5% in Great Island, Moody 2001), but EPC behaviour has been reported, including a significant number of forced EPCs of females (Birkhead et al. 1985; Hatchwell 1988). Recently, the EPP rate for a population of Common Murres, obtained via multi-locus DNA fingerprinting, has been reported at 7.7% (Birkhead et al. 2001). However, to date, no single study has combined extensive behavioural observations with parentage analysis on individuals whose mating history and EPC behaviour was known.

A detailed examination of pair and extra-pair copulation behaviour in Common Murres was carried out over four breeding seasons and is reported in Chapter 2. A banded subpopulation of murres on Great Island, Newfoundland, was observed intensively from

1997-2000. Since behavioural observations exist for many individuals in more than one year, the relevant events in the lives of individuals (e.g., death of a mate, divorce) could be related to specific EPC behaviour patterns. A focus on "who" performed EPCs, when they did so, and whether such EPCs resulted in any EPP permitted me to analyse both who controlled EPC behaviour and paternity outcome and to gain insight into the functions of EPCs for Common Murres. Chapter 3 describes details of the paternity analyses carried out on some of the pairs studied in Chapter 2. Using four microsatellite loci (Ibarguchi et al. 2000), cases of ambiguous chick-parent mismatches are ascribed to either EPP, adoption alloparenting, misidentification, and/or mutation. In Chapter 4, the usefulness of the microsatellites to examine the genetic structuring within and among Common Murres from three ledges on Great Island, Newfoundland (including the ledge at which the behavioural and parentage analyses were carried out) and two ledges areas on Funk Island, Newfoundland was examined. Average and pairwise coefficients of relatedness (R; Hamilton 1964) were estimated and are discussed in the context of kin selection, inbreeding, and philopatry.

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1.2 Co-authorship Statement

I proposed this project for doctoral research and designed all aspects of the behavioural and genetic studies in collaboration with my co-supervisors, Dr. Anne Storey and Dr. William Davidson. I conducted field research with the assistance of eight other observers of murre behaviour over the course of this study. Since many behavioural observations were made by my fellow graduate student, Sabina Wilhelm, whose thesis focuses on other aspects of Common Murre reproductive behaviour, she is a co-author on all manuscripts produced from this thesis. I was solely responsible for the molecular genetic laboratory work, but received assistance with running approximately half of all microsatellite gels from a paid laboratory assistant. I alone analyzed all genetic and behavioural data (i.e., I scored and interpreted autoradiographs and conducted all statistical and genetic analyses). I am the primary author of the manuscripts that follow, presented as thesis chapters, none of which are currently published. Co-authors of Chapters 2, 3 and 4 include Anne E. Storey and Sabina I. Wilhelm, due to their intellectual and practical contribution to the overall project. Also, William S. Davidson is a co-author of the genetic studies described in Chapters 3 and 4.

Chapter 2
Copulation Behaviour in Common Murres: Who is in Control?

2.1 Abstract

The copulation behaviour of Common Murres (*Uria aalge*) was studied on Great Island, Newfoundland, Canada from 1997-2000, to investigate the prevalence and functions of extra-pair copulations (EPCs). Various individuals were observed participating in extra-pair copulations, although the majority of those that successfully performed EPCs did so in one year only. While there was significant variation among years in the amount of pair copulation (PC) behaviours, the overall low frequency of EPCs remained relatively stable over the entire study. Female murres which resisted PCs or EPCs were always able to prevent cloacal contact. As well, there were no multi-male EPC attempts that were observed to have been successful (i.e., resulted in cloacal contact). Copulations initiated by females were more likely to be successful than male-initiated copulations. In general, females that accepted EPC attempts from males were present in the colony more often than females which did not accept EPCs. Female participation in successful EPCs was also related to the stability of their pair bonds: females which divorced over the course of the study had more successful EPCs than females that were in stable pair bonds. Male EPC behaviour was unrelated to pair bond stability. Most females participating in EPCs accepted PCs following their EPCs. It is suggested that behavioural modification of PCs may be required if the function of EPCs is to obtain an extra-pair fertilization (EPF). Most EPCs occurred in the absence of the participants' mates. However, there was no evidence for effective mate guarding of

females by male Common Murres. EPCs by females appeared to serve three non-exclusive functions: 1) obtaining genetic benefits from extra-pair males, 2) facilitating mate change, and 3) ensuring fertility. This study indicates that female Common Murres largely control the outcome and paternity consequences of both pair and extra-pair copulations.

2.2 Introduction

The performance of extra-pair copulations (EPCs) by socially monogamous birds has received considerable attention since the 1980s, when it first became apparent that males obtained copulations with females who were not their social partners (e.g., reviewed in Westneat et al. 1990; Birkhead & Møller 1992, 1998). The plethora of empirical studies on copulation behaviour that followed now show that EPCs in birds are ubiquitous, although there is much variability among species as to whether behavioural observations of EPCs accurately predict rates of extra-pair fertilization (EPF) obtained from molecular analysis of chick paternity (Dunn & Lifjeld 1994; Birkhead & Møller 1995).

Not surprisingly, there has been much discussion of both the costs and benefits of EPCs for males and females (Wagner 1992*a*; Sheldon 1994; Keller & Reeve 1995; Enquist et al. 1998; reviews in Birkhead & Møller 1992, 1998), as well as how such a behavioural strategy in birds has evolved (Ligon 1999). While it is generally accepted that males perform EPCs mainly, but not necessarily exclusively, for the purpose of

maximizing the possibility that their sperm will fertilize an extra-pair female's egg(s), it is less clear how females benefit from EPCs (Birkhead & Møller 1992, 1998). Birkhead (1998*a*) contended that, of the possible hypothesized benefits of EPCs to females, the one with most support to date is the so-called "good genes" theory, i.e., females perform EPCs to receive indirect genetic benefits. Such indirect benefits include not only obtaining good genes (i.e., high quality young) but also increasing the genetic variability of offspring or obtaining viability genes (for a detailed review of genetic benefits see Jennions & Petrie 2000). However, Birkhead (1998*a*) also concedes that, "...for a rather small number of special cases...", there is evidence that females obtain some direct benefits from EPCs (p. 611). These include fertility insurance, acquisition of nutrients, paternal care, and facilitation of change in partner. Procuring such direct benefits and obtaining indirect genetic benefits from EPCs are not necessarily mutually exclusive (Jennions & Petrie 2000). Thus, there may be multiple and different benefits of EPCs for different individuals even of the same species.

Common Murres (*Uria aalge*), a colonial seabird species of the Northern Hemisphere, have been the focus of two studies of extra-pair copulation behaviour (Birkhead et al. 1985; Hatchwell 1988). The life history of this species makes it an interesting subject for such study, as these birds are long-lived, produce only one chick per year, have low divorce rates, and copulate only in the colony (Gaston & Jones 1998). Both studies reported that EPCs occurred frequently, and at comparable rates, in the two colonies observed (Gannet Islands, Labrador, Canada in Birkhead 1985; Skømer Island, Wales, UK in Hatchwell 1988). While these studies clearly described many details of

Common Murre EPCs (e.g., timing and frequency of occurrences, relationship of the operational sex ratio and density in the colony to EPCs), they were unable to (nor was it their intention to) adequately address the issue of the individual circumstances under which EPCs occur, i.e., who performs EPCs, as well as when they do so.

In order to attempt to completely understand the costs and benefits of EPCs for any species, a long-term investigation of both the copulation behaviours and paternity outcomes for marked individuals is required. Such an approach has been recommended to comprehend the large degree of variation among species in levels of extra-pair paternity (EPP), which reflect among-species differences in the frequency of EPCs and or the success rate of EPCs in fertilizing eggs (Petrie & Kempenaers 1998). Several studies have combined extensive behavioural observations of banded individuals with paternity analyses (e.g., Johnsen et al. 1998; Ramsay et al. 2000) and have described circumstances related to (or, just as importantly, unrelated to) EPC behaviour in the particular species examined. However, the frequencies of EPCs or EPP in one population and, by extension, the costs and benefits of EP activity will not necessarily be identical in other populations of the same species (Griffith et al. 1999; Petrie & Kempenaers 1998). Indeed, there has been temporal variation in EPC behaviour and EPP rates reported within a population of Red-winged Blackbirds (*Agelaius phoeniceus*) over a 5-year period (i.e., P.J. Weatherhead, pers. comm. cited in Petrie & Kempenaers 1998; Weatherhead et al. 1994).

The current study describes the copulation behaviours of a group of individually-marked Common Murres studied from 1996-2001. The behavioural analyses which

follow focus on the years from 1997-2000, as a low number of murres were banded in 1996 (i.e., behavioural observations for many individuals were incomplete in that year), and the paternity analyses (detailed in Chapter 3) were conducted for chicks from 1996-1999. The general patterns of both pair and extra-pair copulations are examined across years. As well, specific case studies of individuals which participated in successful EPCs over the course of the study are described in order to achieve a more thorough understanding of the circumstances under which EPCs occur in Common Murres.

2.3 Methods

2.3.1 Study Area

A group of Common Murres breeding on a cliff ledge (measuring approximately 1.6 m X 2.5 m wide) on the southeast end of Great Island (47°11'N, 52°49'W), Newfoundland, Canada was studied (Figure 2-1). Great Island is one of four islands in the Witless Bay Ecological Reserve, and this ledge had been an established breeding site for murres since at least the 1980s (Cairns et al. 1987, 1990). A permanent wooden blind, with one-way glass for viewing the murres, was located at the peripheral edge of the site (since 1984) and demarcated the western end of the breeding ledge. A second ledge, to the south of the blind and study ledge, demarcated one side of the plot. The other end of the study ledge continued to the base of another cliff, upon which more murres bred. The northern edge of the plot was open to the ocean; this was the direction of arrival and departure of murres to and from the ledge.

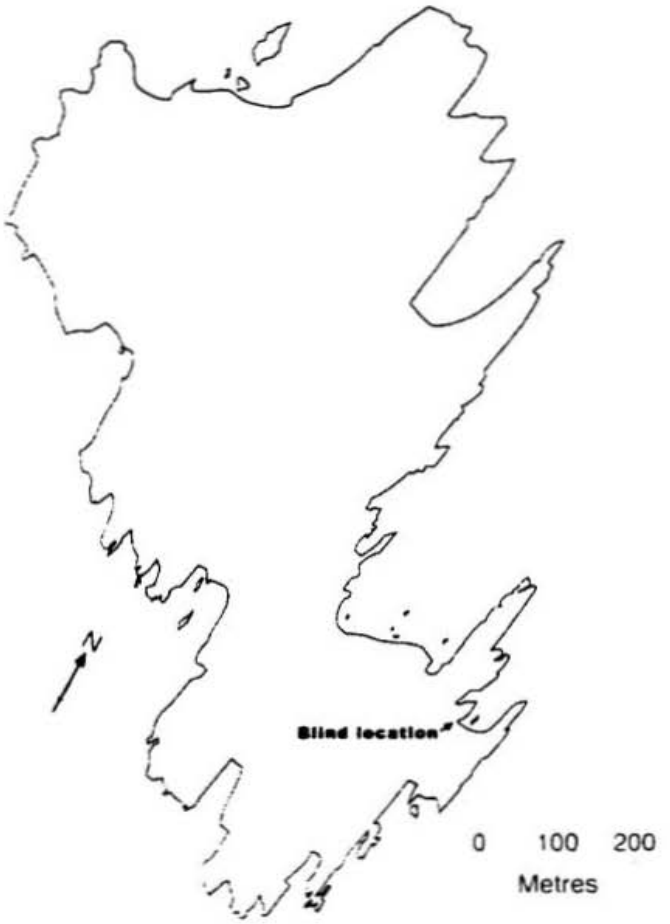

Figure 2-1. Map of Great Island (47°11'N, 52°49'W), Newfoundland, showing the approximate location of the study blind on the "DC Ledge".

Approximately 3000 pairs of breeding murres were on Great Island during the 1980s (Cairns et al. 1989). However, observations since the mid-late 1990s indicate that the murre population is expanding on Great Island (S. Wilhelm, A. Storey, pers. comm.)

and on nearby Green Island (W. Montevecchi, pers. comm.). The study ledge contained approximately 35 breeding sites, and the number of murre pairs present at the site during pre-laying (i.e., the portion of the breeding season prior to the median laying date for that year) has fluctuated slightly over years (from approximately 28 to 40 pairs).

2.3.2 Number of Breeding Pairs at the Study Site

Banding of Common Murres with Canadian Wildlife Service (CWS) and colour bands began in 1996 under the direction of A.E. Storey at the Great Island study site and is ongoing. There were several murres at the study site in 1996 that had been previously banded by Cairns, et al. (1987, 1990). From 1996-2000, there has been a gradual increase in the number of banded individuals present. Behavioural observations were recorded for both banded and known unbanded murres. Observations of unbanded murres were included only for those cases in which there was a high degree of certainty of the individuals' association with a particular breeding site, usually by means of identification by a unique physical feature (e.g., bridling of one pair member and not the other), or by identification through a unique spray pattern of picric acid, applied with a toy water gun or blown from a flask (via PVC tubing) placed permanently in the site. Within and between-year site fidelity is a feature of murre breeding behaviour (Harris et al. 1996) and several behavioural studies of Common Murres have used unmarked birds identified by breeding site associations (Birkhead et al. 1985; Hatchwell 1988; Davoren 2001).

Catching of murres for banding and blood sampling was done by extending a noose pole from behind a canvas partition onto the ledge during pre-laying and mid-to-late chick rearing. Particularly in the pre-laying period, the process of catching individuals was frequently followed by many murres leaving the plot for a short period of time (several minutes to an hour or more). Catching effort in the pre-laying period typically involved only 1-2 hours per day over the course of several days. Following cessation of catching each day, murre attendance normalized, and behavioural observations obtained on these "catching" days were not excluded from analyses. In general, catching was more successful during chick rearing, as adults with chicks left the ledge less frequently during the disturbance. Behavioural observations continued throughout catching in the chick-rearing period when possible, in order to confirm the identity of the chick and to determine if the colour-marked chicks returned to their parent(s) after banding. In five years of catching, all chicks have been reunited successfully with their parents.

Behavioural data were collected for 29 breeding pairs in 1997, 33 pairs in 1998, 32 pairs in 1999, and 36 pairs in 2000. Behaviour from 1996 was not analyzed, as there was a comparatively low number of individuals (n = 26) identified in that year. However, blood and or feather samples were taken from adults and chicks beginning in 1996, and these individuals were included in the analysis of chick paternity (Chapter 3).

2.3.3 Observations

Observations were made by nine different observers from 1997-2000, four of whom were present in more than one year. High inter-observer reliability was obtained by having sets of two observers simultaneously watch the murres early in the season and agree upon the observation criteria needed to categorize particular behaviours. New observers were always paired with an experienced observer initially. Blind watches ranged in duration from 81 minutes (occurring in 1999) to 970 minutes (occurring in 1998) during the pre-laying season, and typically began at dawn (approximately 0500 h) and lasted until dusk (approximately 2030 h). Behavioural observations were recorded continuously. There was a tendency for observation watches to be shorter in 1999 than in other years; many terminated around 1200 h due to low (or no) attendance of murres at the site. As a result, attendance data analyses comparing different years were restricted to the morning period until noon (i.e., the forenoon). For the pre-laying period, there were a total of 15 observation days (116 h) during which behaviour was recorded and analyzed in 1997 (between May 15- June 5), 13 days (140 h) in 1998 (between May 6- May 27), 16 days (161 h) in 1999 (from May 11- June 4), and 15 days (170 h) in 2000 (from May 11- June 2). Variation in the starting date each year was due to the inability to access Great Island any earlier in the spring (i.e., ice and/or sea conditions prevented landing on the island).

2.3.4 Behaviour Recorded and Terminology

For each observation day, the attendance of individuals at the ledge was recorded in 30 minute blocks from the beginning of the watch into late afternoon. All arrivals and departures for known individuals within each 30 minute block were noted, and a scan of the site was made at the beginning of each 30 minute period to determine which birds were present. In the early part of 1997, attendance records of individuals by site were reliable, but the attendance of pair members by sex was not (i.e., for several unmarked pairs it was possible to determine male and female presence only when both individuals were at the site together). Thus, it was not possible to analyze attendance for this year by sex. As well, there were 3 days in 1997 (May15, 16, 18) and 4 days in 1998 (May 6-8, May14) for which there were no reliable attendance records. As the number of spot checks per day (or forenoon) frequently varied due to catching activity or attendance records beginning later than 0500 h, each individual's attendance was quantified by the proportion of checks in which he or she was present relative to the total number of spot checks in each year.

The main behaviours recorded for each individual present at the site were copulation behaviours. The definitions in this study for various types of copulations differed somewhat from the terminology used in two prior studies of Common Murre reproductive behaviour (i.e., Birkhead et al. 1985; Hatchwell 1988). Specifically, I eliminated the categories of "forced" vs. "unforced" copulation, where the former term implies that the female resisted the copulation but was not always successful at

preventing cloacal contact (see Section 2.5.3). In this study, observers never recorded a resisted copulation attempt that was successful, i.e. resulted in cloacal contact between the male and female. The following terminology was used in the current study:

(1) Pair Copulation (PC) Success: A copulation between members of an established pair, or, if the individuals were unpaired, a copulation between a male and female that later became an established pair during that year, that resulted in successful cloacal contact. Successful PCs were almost always accompanied by the female emitting the characteristic copulation call during copulation (Gaston & Jones 1998; AES, SIW, CJW, pers. obs.), and were typically terminated by the female standing up, although males occasionally terminated the copulation by dismounting.

(2) Pair Copulation (PC) Attempt: A copulation activity between members of an established pair (or, if unpaired, between individuals that became an established pair later that year) that did not result in cloacal contact, i.e., was unsuccessful. Unsuccessful PC attempts were less often accompanied by the female's copulation call, and the lack of cloacal contact was seemingly due to either: (a) the female standing before the male could become appropriately positioned (i.e., if the female crouched, she did not remain in that position long enough for cloacal contact to occur) or (b) an inability for the pair to temporally coordinate their activity (i.e., the female's crouching and raising her tail, and the male's bending his lower body to reach the female's cloaca at the same time).

(3) Extra-Pair Copulation (EPC) Success: A copulation between two individuals who, if one or both of them was mated to another individual, were not an established pair or, if both were unpaired, did not become an established pair, that resulted in cloacal contact. Like PC successes, successful EPCs were frequently accompanied by the female's call during copulation, and most were terminated when the female stood up.

(4) Extra-Pair Copulation (EPC) Attempt: A distinction is made between two types of EPC attempts-

   a) Single-male EPC Attempt: A copulation activity between two individuals who, if one or both of them was mated to another individual, were not an established pair or, if both were unpaired, did not become an established pair, that failed to result in cloacal contact. This failure was almost always due to the female simply standing up.

   b) Multi-male EPC Attempt: A copulation activity between one individual and two or more males (if the recipient of the attempt was paired, neither of which were her mate). These attempts appeared to be resisted by females; females would stand immediately in response to the attempt, move away from the males, and/or peck them. Unless otherwise stated, the general term "EPC attempt" always refers to single-male EPC attempt (see Section 2.4.5 for rationale of this decision).

(5) Initiator: The individual or individuals that appeared to instigate the copulation event (i.e., PC or EPC activity). The initiator could be (a) male: determined if

the male approached the female and attempted to mount her; this action was often accompanied by the male's crow call before the copulation started (Gaston and Jones 1998). (b) female: determined if the female approached the male, and or crouched by him, and or made the signature copulation call, or (c) both: determined if both the male and female fulfilled the requisites for male and female initiators in a simultaneous manner.

(6) "Stable" vs. "Unstable" Pair bond: A pair was labeled stable if, from 1997-2000, the pair did not experience a divorce (the termination of a pair bond due to the departure from the site of one mate who was determined to be alive following the pair's separation). A pair or individual was labeled unstable if either member of the pair experienced a divorce. If labeled unstable in one year of the study, the label was applied to the individuals of this pair for both previous and subsequent years, as it is possible that these individuals may be more likely to experience a subsequent divorce.

The general terms "PC activity" and "EPC activity" refer to both single-male attempts and single male successes combined. "Total copulation activity" refers to all PC and EPC activity, including multi-male EPC attempts.

Egg laying and chick hatching dates were recorded (or, for unobserved laying dates, were back-calculated by subtracting 33 days from the observed hatch date; Gaston and Jones 1998) for each female when possible. In addition, once pairs had chicks,

measures of parental care were recorded including the number of visits that each parent made to the site and the number of fish brought to the chick by each parent.

2.3.5 Data Analysis

All data were analyzed using SPSS (version 10.0 for Windows) statistical software. Parametric analyses were conducted whenever possible, and assumptions for normality and equality of variances were always examined (e.g., option for homogeneity of variance selected and Levene's statistic for unequal variance checked). If analyses indicated that parametric assumptions were violated, appropriate nonparametric tests were performed. Multiple Regression and/or Discriminant Analysis were deemed inappropriate for these data, as the sample sizes for each year were small in relation to the number of variables to be examined (McGarigal et al. 2000). Parametric analyses used included One-Way ANOVA (Compare Means- One-way ANOVA), Pearson's product-moment correlation (Correlation- Bivariate), One-way Repeated Measures ANOVA (GLM-Repeated Measures), Multivariate Repeated Measures ANOVA (GLM- Repeated Measures using more than one measure), and Univariate ANOVA (GLM- Univariate). Post-hoc tests used were Tukey's Honest Significant Difference or Dunnett's test for unequal variances (as recommended in Gardner, 2001). If post-hoc multiple pairwise comparisons were conducted following significant repeated measures ANOVAs, Bonferroni adjustments were employed. Non-parametric tests included chi-square analysis (Crosstabs procedure, Yate's correction for small sample sizes and df = 1 applied when indicated), Kruskal-Wallis and Mann-Whitney tests (Nonparametric- K and 2

Independent Samples), and Kolmogorov-Smirnov tests for departure of data from uniform and normal distribution (Nonparametric- 1 Sample). The type of procedure utilized is reported with the pertinent results (Section 2.4).

When proportions were analyzed (e.g., per cent attendance), data were arc sine transformed to meet the assumptions of normality (Sokal & Rohlf 1981). Means and standard deviations are presented when the data were mainly analyzed with parametric statistics, while medians and ranges are reported for data that were *a priori* determined to be more appropriately analyzed by nonparametric statistics.

For many of the behavioural analyses that follow, data were treated as independent for each year of the study. Of course, the majority of individuals present in the study site each year were the same ones present in the previous and subsequent years. Thus, the behavioural data are not independent across years, because much of the behaviour is performed by the same murres in more than one year. Given these facts, it might be recommended that a repeated measures design be used, effectively eliminating the pairs or individuals for which there are data missing in one or more years. While such an approach has merit, I feel that it would be unfair in the context of this study to limit the data to such analyses, as many behaviours, particularly those which are relatively infrequent, or which are performed by specific individuals in specific years (such as EPC activity), would be lost. As a result, the depiction of the behaviours performed by the group within each year could be inaccurate. Hence, for several analyses, data are

considered independent across years in order to capture the full scope of behaviour exhibited in different years by individuals, pairs and the group as a whole.

Obviously, this type of analysis confounds several factors: 1) individual differences in the behavioural propensity to engage in copulations (e.g., some males and females may be predisposed to higher rates of copulation activity due to physiological differences), 2) external or environmental factors that affect the overall levels of copulation in the colony (e.g., high attendance on the ledge may provide social facilitation of copulation behaviours), and 3) unique individual factors that might increase or decrease the proclivity of a particular individual to engage in copulation activity (e.g., social factors such as mate loss, or increasing age of an individual). Thus, in order to examine whether treating the data in a repeated-measures manner would produce results different from those obtained when data were treated as independent, the copulation behaviours of a subset of pairs (n = 13) were analyzed separately in a Repeated Measures ANOVA. Criteria for inclusion in this subset was that at least one member of the pair had at least one copulation behaviour recorded in all four years of the study. The pairs which met these criteria were: 1, 2, 4, 6, 9, 10, 11, 12, 16, 20, 24, 25, and 29. The repeated-measures results (Section 2.4.11) essentially emulated the latter analyses, supporting the notion that treatment of the data as independent among years was fair and reasonable. In fact, analyzing data only for the same pairs over time actually eliminates just the first confounding factor described above, i.e., the individual variability in the propensity to copulate that might exist. As the other factors would likely vary across years, they must be analyzed separately, where possible, and considered carefully in the interpretation of

any yearly differences in copulation rates, but they cannot be eliminated by either study design or statistical analysis.

Within each year, the distribution of the number of copulations performed by pairs (for PC activity) and by individual males and females (EP activity) was analzyed for departure from normality in order to account for variation among individual birds. If these copulations are not normally distributed, this would suggest that the majority of copulations were performed by certain pairs or individuals. Normal distribution of these copulations, however, would indicate that variation among pairs or individuals is minimal.

2.3.6 Copulation Behaviour and Date with respect to Egg Laying

In order to standardize copulation activity with respect to yearly differences in laying dates, I examined behaviours from a restricted period corresponding to the two attendance peaks, during which both male and female attendance was high on the ledge, immediately prior to the first egg being laid (Wilhelm et al. 2000: Figure 2-2). As date with respect to median egg-laying date likely affects copulation behaviour and paternity outcome (Hatchwell 1988), restricting most analyses to these attendance peaks will minimize such effects. For the years 1997, 1998, and 2000, the first of these two attendance peaks begins an average of 12 days prior to the first egg laid on the ledge (1997 -14 days; 1998-12 days; 2000- 11 days), while the second begins an average of 7 days prior (1997-9 days; 1998- 6 days; 2000- 7 days). Thus, during this "Peak period"

(i.e. two peaks combined), it is likely that the majority of females are fertile (Birkhead et al.1985; Hatchwell 1988). In 1999, there was no clear pattern of attendance peaks (Wilhelm & Storey, in prep.), so peak dates were imposed on the data for this year, using the mean number of days prior to the beginning of egg laying as reported above. The entire peak period for 1997-1999 inclusive is 6 days long, while it is 5 days long in 2000 due to the first attendance peak lasting only 2 days.

For all years except 2000, copulation activity occurring outside the Peak period was also examined. Data for the period before the Peak period were obtained in 1997 (n = 1 day) and 1998 (n = 3 days). There were no behavioural observations made during this "Before Peak" period in 1999 (or 2000), as observers could not be present at the study site during this time. Data are more complete for the "After Peak" period, consisting of 8 days in 1997, 3 days in 1998, and 8 days in 1999. The pattern of copulation behaviour for the "Before Peak" period can only be analyzed reliably for 1998; however, changes in behaviour from the Peak period to the "After Peak" period are described for all years.

Unless otherwise indicated, the behavioural analyses were carried out for behaviours occurring during the Peak period in each year.

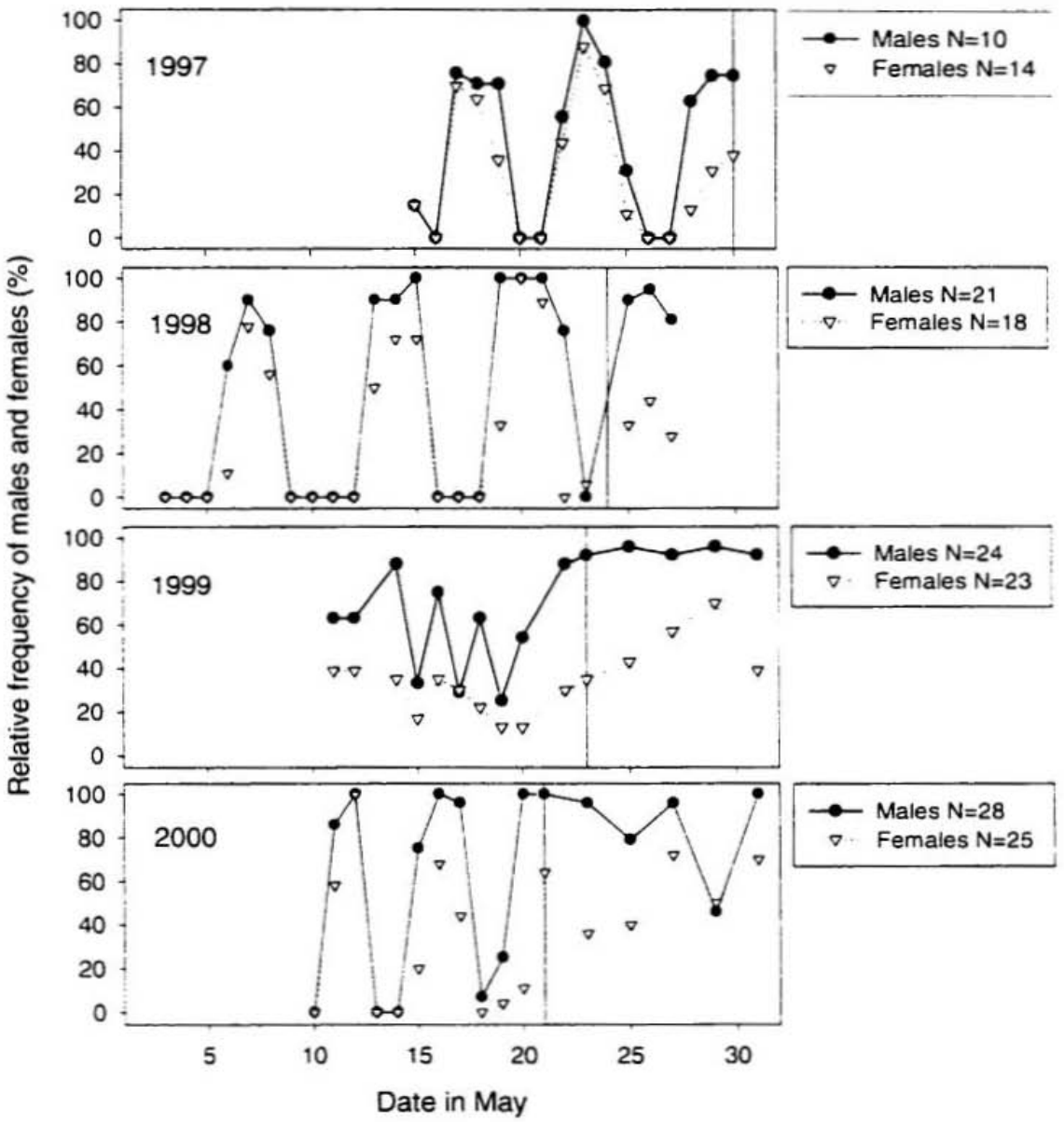

Figure 2-2. Attendance peaks for male and female Common Murres during the pre-laying periods of 1997-2000. Vertical line indicates date that first egg was laid in the ledge (used with permission from Wilhelm and Storey, submitted).

2.4 Results

2.4.1 Comparison of Copulation Behaviour Across Years

Comparisons of total daily copulation activity rates from within and outside the Peak periods from 1997-1999 show that significantly more total copulation activity per day occurred during Peak days than either before or after this period (Mean ± SD: Peak (n = 18), 42.4 ± 37.7; Non-Peak (n = 23), 21.7 ± 17.1; $z = -1.99$, $p<0.05$, Mann-Whitney test). If 1999 (the year with imposed peak attendance periods) is removed from this analysis, the increased levels of copulation activity within vs. outside the Peak period are even more pronounced (Mean ± SD: Peak (n = 12), 56.8 ± 38.2; Non-Peak (n = 15), 20.4 ± 18.8; $z = -3.1$, $p<0.01$, Mann-Whitney test).

2.4.2 Yearly Differences in Mean Daily Copulation Activity

There was a significant difference in the number of observation hours per day during the Peak period of 1998 (total of 75.5 hours) and 1999 (total of 37.2 hours; $F_{[3, 19]} = 4.18$, $p<0.02$; Tukey's post-hoc test). As stated previously (Section 2.3.3), this difference was due to low and erratic attendance patterns of the murres in 1999 which resulted in behavioural recording typically terminating at noon during the Peak period of that year. This difference in observation duration among years was controlled for by either converting the frequency of behaviours into hourly rates, or by examining behaviours in the forenoon only (e.g., Section 2.4.3).

There was a significant difference in the daily mean PC Attempts/hour (i.e., unsuccessful pair copulations) across years ($\chi^2_{(3)}$=13.3, p<0.01; Kruskal-Wallis test, Table 2-1). This effect is largely accounted for by differences between 1998 and 1999 (z = -2.9, p<0.01, Mann-Whitney test), with significantly more unsuccessful PCs occurring in 1998. There were no significant differences among years for the mean daily total PC successes, multi-male EPC attempts, single-male EPC attempts or successes, or total copulation activity.

2.4.3 Yearly Differences and Site Attendance

To ensure that the above differences in total copulation activity among years were not due only to more birds attending the site in a particular year, an analysis was conducted based on individual attendance at 30-minute intervals beginning at first light and terminating at noon. Noon was chosen as the termination point due to: 1) the fact that more than half of the daily copulation activity occurs prior to noon (Figure 2-3), and 2) most observations for 1999 were carried out in the morning, as birds were rarely in attendance in the afternoon for that year only. The attendance records were examined to determine the maximum number of individuals present during any given spot check on

Table 2-1. Mean rates/hour of total copulation activities occurring per day during the Peak periods of 1997-2000 (Mean ± SD; total number of days = 23).

	YEAR			
	1997	**1998**	**1999**	**2000**
	(n=6)	**(n=6)**	**(n=6)**	**(n=5)**
PC Successes	1.78±0.93	2.31±0.91	1.22±1.40	1.96±1.80
PC Attempts*	1.16±0.87	2.43±1.35	0.11±0.17	0.91±0.94
EPC Successes	0.19±0.20	0.21±0.12	0.40±0.55	0.38±0.17
Single-Male				
EPC Attempts	0.56±0.42	0.43±0.25	0.40±0.24	0.54±0.30
Multi-Male				
EPC Attempts	0.27±0.44	0.06±0.07	0.21±0.24	0.15±0.23
Total Activity	3.97±1.94	5.42±2.02	2.34±1.65	3.93±2.44

* significant yearly difference, $p<0.05$

each day, as well as the maximum number of males and females present (excluding 1997). There were no significant differences in either the maximum number of individuals present per day for the Peak period among years ($\chi^2_{(3)} = 4.66$, ns, Kruskal-Wallis test), nor were there differences in the maximum number of males or females present. However, there was a trend in the data showing fewer individuals present in 1999 than in other years.

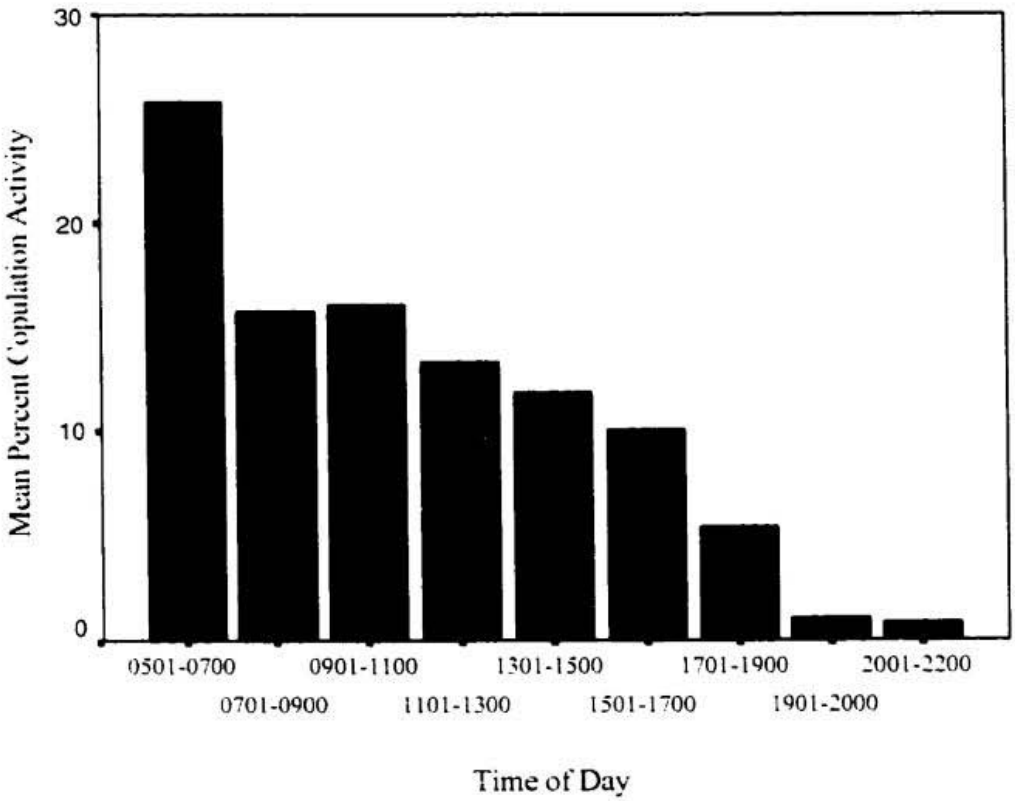

Figure 2-3. The distribution of mean percent copulation activity across the observation day in Common Murres during the Peak periods from 1997-2000.

2.4.3.1 Were among-year differences in mean daily copulation activity affected by the maximum number of individuals present at the site in the forenoon across years?

In order to gauge whether there was an effect of attendance patterns on copulation activity among years, total daily copulation activity (the sum of all successful and unsuccessful PCs, EPCs, and multi-male EPC attempts) was divided by the maximum number of individuals present in the forenoon (as well as by the maximum number of females and males). There was a significant difference in the mean daily copulation activity/maximum number of individuals present across years ($F_{(3,16)} = 3.84$, $p<0.03$; One-way ANOVA). Post-hoc analysis shows that this difference is due to more copulation activity/maximum number of individuals (a conservative measure of the true activity per individual) in 1998 than 1999 (Mean = SD: 2.4 = 1.4; 0.8 = 0.5, respectively; Tukey's HSD, $p<0.05$). Similarly, there was more average daily copulation activity per maximum number of males in 1998 (Mean = SD: 4.2 = 2.3) than in either 1999 (Mean = SD: 1.4 = 0.7) or 2000 (Mean = SD: 1.7 = 0.7; $F_{(2,13)} = 5.94$, $p<0.02$, One-way ANOVA, Tukey's HSD). This was not the case for females, as there were no differences among years ($F_{(2,13)} = 0.65$, ns).

2.4.3.2 Was there a relationship between the maximum number of individuals present and the types of copulation activity observed?

Various types of copulation activity might relate differently to the sex and number of individuals present at the site. Bivariate correlation analyses (Pearson's r) were conducted across years on 1) the maximum number of individuals present in the forenoon

and the frequency of different types of daily copulation activity, and 2) the ratio of the maximum number of females to males in the forenoon and these types of copulation activity. Both the maximum number of individuals and the ratio of females to males correlated strongly with pair activity (PC attempts and PC successes), but were unrelated to extra-pair activity (Table 2-2). Similarly, there was a significant positive partial correlation between the maximum number of females present and both the number of PC attempts and PC successes, controlling for the maximum number of males; i.e., the more females present, the more PC activity (Table 2-2). This relationship did not exist for males, when the maximum number of females present was controlled for. Interestingly, there were negative, albeit non-significant relationships, between the number of females and total EPC attempts, as well as total EPC successes, suggesting that EPCs tended to occur when there were fewer females present. This relationship did not exist for males.

Multi-male EPC attempts were not significantly correlated with either measure of attendance (maximum individuals, $r_{(20)} = 0.06$, ns; ratio max. female:male, $r_{(16)} = 0.04$, ns; max. number females, partial $r_{(13)} = -0.25$, ns; max. number males, partial $r_{(13)} = 0.38$, ns).

Table 2-2. Pearson's correlation coefficients for the daily frequency of various types of copulation activity and 1) the maximum number of individuals present at the site in the forenoon, 2) the ratio of maximum female to males per forenoon and partial correlation coefficients for different types of copulation activities and 3) the maximum number of females present (controlling for the maximum number of males), and 4) the maximum number of males present (controlling for the maximum number of females).

		PC Attempts	PC Successes	EPC Attempts	EPC Successes
Max. Individuals	$r_{(20)}$	0.67**	0.77**	-0.21	-0.01
Ratio Max. Females:Males	$r_{(16)}$	0.65**	0.63**	-0.45	-0.19
Max. Females controlling for max. males	$r_{(13)}$	0.59*	0.53*	-0.44	-0.31
Max. Males controlling for max. females	$r_{(13)}$	-0.19	0.09	0.38	0.31

* $p<0.05$

** $p<0.01$

2.4.4 Copulation Success, Duration and Cloacal Contacts

Overall, 60% of total PC activity (381/639 copulations for which outcome was determined) resulted in cloacal contact, while only 32% of total EPC activity (45/143 single EPC copulations for which outcome was known) did so ($\chi^2_{(1)} = 36.3$, $p<0.01$, Yate's correction applied). Successful copulations, i.e., those in which at least one cloacal contact was recorded, lasted significantly longer than unsuccessful attempts ($z = -13.2$; $p<0.01$, Mann-Whitney test; Table 2-3). However, there was no difference in copulation duration between successful EPCs and PCs ($F_{(1, 408)} = 0$, ns). Similarly, there was no difference in the number of cloacal contacts made in successful EPCs and PCs ($z = -0.11$, ns, Mann-Whitney test). Year had no effect on copulation duration ($F_{(3,606)} = 0.841$, ns), or the number of cloacal contacts achieved ($F_{(3,--8)} = 1.58$, ns).

Table 2-3. Number of cloacal contacts achieved and copulation duration for unsuccessful and successful extra-pair and pair copulations (N=782) from 1997-2000.

Copulation Type	Success?	# Cloacal Contacts	Copulation Duration (s)
Extra-Pair	**YES**	2.5±2.8 (n=45)	26.4±28.5 (n=41)
	NO	0 ± 0 (n=98)	5.7±2.8 (n=21)
Pair	**YES**	2.2±2.0 (n=381)	26.4±27.4 (n=369)
	NO	0 ± 0 (n=258)	7.9±3.6 (n=141)

2.4.5 Extra-Pair Success and Presence of Mate

A subset of EPCs (successes and attempts; 95/143 = 66%) occurring during the Peak period from 1997-2000, for which I knew both the copulation outcome and whether the mates of the extra-pair individuals were present, were examined. Most EPC activity (86/95; 90.5%) occurred when one mate or both mates of the copulating pair were absent from the ledge (Table 2-4). Few EPC attempts were ultimately successful; only 33/95 (34.7%) resulted in cloacal contact. Of the 33 successful EPCs, 21 (63.6%) occurred in the absence of one mate, while nine (27.3%) occurred in the absence of both mates of the (EP-) copulating pair. Males were more likely to attempt EPCs when their female mates were absent (71/95 cases; 74.7%). In fact, during the Peak periods, there were no cases in which a male attempted an EPC while his mate was present on the ledge. However, the outcome of EPCs did not appear to depend on the absence of mates in the ledge; for example, of the 34 EPCs observed when both mates of the copulating pair were away from the colony, only nine (26.5%) were successful. Females whose mates were absent did receive more EPC attempts than females whose mates were present (male absent: 49/95 vs. male present: 16/95), however, females did not seem to accept more EPCs when their mates were absent (14/49) versus when they were present (3/16; $\chi^2_{(1)} = 0.201$, ns; see Section 2.5.1).

No multi-male EPC attempts were observed to result in cloacal contact, either during the Peak period (n = 37), or outside the Peak period (n = 47). Most multi-male EPC attempts were directed at birds as they landed in the study plot. For the cases in

which the recipient of the multi-male EPC attempt was a marked individual (n = 55: 25 within Peak and 30 outside Peak period). 89% (49/55) were female. For 19 (of 25) multi-male EPC attempts that occurred during the Peak period, for which the presence or absence of the female's mate was known, 63% (12/19) occurred when the male was absent.

None of the multi-male EPC attempts observed lasted longer than 5 seconds before the recipient of the attempt stood and/or moved away. This is a copulation duration similar to that recorded for both unsuccessful pair and single-male extra-pair copulation attempts. Thus, it seems highly probable that cloacal contact is never made during these multi-male EPC attempts.

2.4.6 Interruptions

Interruptions were recorded whenever a copulation attempt or successful copulation was stopped due to the activity of another bird that was directed specifically at the copulating pair (e.g., cessation of copulation due to apparently inadvertent interference from another bird was not scored as an interruption). Interruption of copulations has been suggested to be a mechanism whereby a male might stop an EPC attempt on his mate, or might obtain an EPC by displacing the copulating male (Hatchwell 1988). Overall, very few copulations were interrupted in the Peak period when the numbers of birds present in the plot were the highest: only 26/975 copulations

Table 2-4. Number of single-male EPC events (attempts + successes) and EPC successes occurring in the presence and absence of mates (N = 95).

Mate attendance status		Total Number of EPCs (attempts + successes)	Number of successful EPCs (% all EP activity)
Both mates absent		34	9 (26.5%)
Female mate absent	Total	37	16 (43.2%)
male mate present		7	0
male mate unknown		30	16
Male mate absent	Total	15	5 (33.3%)
female mate present		0	0
female mate unknown		15	5
Female mate present. male unknown		0	0 (0%)
Male mate present. female unknown		9	3 (33.3%)
TOTAL		95	33 (34.7%)

were interrupted (2.7%). Only 2/26 interruptions occurred during EPCs; neither of these was an interference by a mate of the extra-pair copulating individuals. Of the 24 PC interruptions, there was no case where the interrupting male successfully replaced the female's mate and obtained an EPC (*cf.* Hatchwell 1988). There was no significant difference in the number of interruptions among years ($\chi^2_{(3)} = 3.40$, ns).

2.4.7 Temporal Patterns of Copulation Activity

All copulations recorded daily in the Peak period across years were divided into nine 1 hour, 59 minute time categories for observations made from 0501-2100 h. There was significant clumping of copulation activity, with 71% of all daily copulation activities occurring before 1300 h, and over 25% occurring in the first 2 hours of observation (0501-0700 h). Since 1999 observations were made primarily during this morning period, data from this year were eliminated and comparison of the observed activity distribution across the day was still significantly non-uniform ($\chi^2_{(8)} = 400.2$, $p<0.01$; n = 973 copulations; Figure 2-3).

There was no evidence of an increase closer to egg laying in the average daily total copulation activity rate across the Peak period when all years were examined together ($F_{(5, 17)} = 0.78$, ns; One-way ANOVA), or when years were examined separately (for departure from uniform distribution: 1997, z = 1.20, ns; 1998, z = 0.91, ns; 1999, z = 0.73, ns; 2000, z = 1.10, ns; Kolmogorov-Smirnov tests). In general, there was a high

degree of variation in the amount of copulation activity performed on a given day during the Peak period across years.

2.4.8 Pair Copulation Rates Differ Among Years

Both successful and unsuccessful pair copulation attempt hourly rates per marked pair were significantly different among years in the Peak period (Table 2-5). Specifically, successful pair copulations rates were significantly higher in 1998 than in 1999 ($\chi^2_{(3)}$ = 11.9, $p<0.01$; Kruskal-Wallis test: $z = -3.05$, $p<0.01$). For unsuccessful pair copulation attempts, rates were lower in 1999 than in all other years, and higher in 1998 than in all other years ($F_{(3, 126)} = 25.8$, $p<0.01$; Dunnett's T3 post hoc tests for unequal variances). Single-male EPC attempts and successful EPCs rates were examined separately per number of marked females and males observed each year during the Peak period (Table 2-5). Unexpectedly, there were no significant differences in either measure of EP activity, indicating that, unlike pair copulation activity, extra-pair copulation attempts and successes existed at relatively low and consistent levels across years.

In order to determine if there was significant variation among pairs in the number of copulations performed within the Peak period, I analyzed the distribution of PCs (attempts + successes) within each year. For each year, with the exception of 1999, the number of PCs were normally distributed among pairs (Kolmogorov-Smirnov tests for departure from normal distribution: 1997: $z = 0.84$, ns; 1998: $z = 0.67$, ns; 1999: $z = 1.53$,

p<0.02; 2000: z = 0.59, ns). This was not the case for the distribution of EP activity for both males and females, which was non-normal in each year (Section 2.4.12) .

2.4.9 PC Success Rates, but not EPC Success Rates, Relative to Total Attempts Differ Among Years

The proportion of all pair copulation activities (total of successful and unsuccessful copulations) that resulted in successful cloacal contact was highest in 1999 (91 ± 16%) and lowest in 1998 (44 ± 26%). The effect of year on this proportion of PC success pair was statistically significant ($F_{(3, 103)} = 11.8$, $p<0.01$; One-way ANOVA, proportions arc-sine transformed). Proportionately more PC activity was successful in 1999, the year with the lowest PC activity levels, than in 1997 (59 ± 30%), 1998, and 2000 (67 ± 26%), while there was lower proportionate PC success in 1998 than in 1999 and 2000 (Tukey's HSD, all $p<0.05$).

The proportion of successful EPCs to total attempts per male (i.e., EPC successes/(EPC successes + EPC attempts)) showed no significant difference across years ($F_{(3,3^{-})} = 0.64$, ns; proportions arc-sine transformed). Similarly, the mean proportion of successful EPCs to total attempts per female was also not significantly different among years ($F_{(3,3^{-})} = 2.49$, ns; proportions arc-sine transformed). Overall, for both males and females, 27 ± 38% of total EP activities resulted in successful copulation. However, there was a high degree of variation in whether EPC attempts were ever successful for any given individual, because successful EPCs involved a small number of males and females each year.

2.4.10 Copulation Activity Occurring Outside the Peak Period

In 1998, there was a trend for PC activity to increase from the "Before Peak" period to the Peak period, followed by a subsequent decrease in the "After Peak" period (e.g., mean daily successful PC copulations: $F_{(2,9)} = 3.27$, $p=0.09$, Figure 2-4*a*). However, there was a simultaneous significant decrease in the mean number of successful EPCs per day from the "Before Peak" to the "After Peak" period during this same time ($F_{(2,9)} = 7.06$, $p<0.02$, Tukey's HSD post-hoc test: Figure 2-4*b*). This suggests that the "Before Peak" period in this year, which began 19 days prior to the first egg laid, may have been characterized by higher levels of successful EP activity than those observed later in that breeding season. There was no significant change in the number of unsuccessful EPC attempts from the "Before Peak" to "After Peak" periods in 1998.

Extra-pair activity showed the general pattern of decreasing from the Peak period to the "After Peak" period in all years, although the decreases were not statistically significant. Similar statistically non-significant decreases in PC activity from the Peak to "After Peak" period were seen in 1997 and 1998. In 1999, however, PC activity increased from the Peak to "After Peak" period. This effect was statistically significant for unsuccessful PC attempts (Mean ± SD: Peak (n = 6 days), 0.8 ± 1.3; After Peak (n = 8 days), 9.3 ± 7.1; $z = -2.88$, $p<0.01$, Mann-Whitney test).

Table 2-5. Hourly rates of copulation behaviours per marked pair (or marked individual)(X 100) during the Peak period across years (Mean X 10^2 ± SD X 10^2; N=130).

	YEAR				
	1997 (n=29)	**1998 (n=33)**	**1999 (n=32)**	**2000 (n=36)**	**Overall**
PC Successes (per pair)	6.26± 5.52[a,b,c]	7.26±5.45[b]	3.78±5.95[c]	5.21±3.94[a,b,c]	5.61±5.33**
PC Attempts (per pair)	4.33±4.41[a]	8.19±5.37[b]	0.42±0.99[c]	2.55±2.59[a]	3.85±4.66**
EPC Successes (per female)	0.59±1.59	0.32±0.10	0.59±1.77	0.98±5.21	0.63±3.00
EPC Attempts (per female)	0.47±1.43	0.92±2.00	1.26±1.93	1.04±2.97	0.94±2.19
EPC Successes (per male)	0.23±0.75	0.56±1.37	0.17±0.66	1.04±3.05	0.53±1.83
EPC Attempts (per male)	1.23 ±2.52	1.00±1.93	0.76±2.18	1.27±2.87	1.07±2.39

**$p<0.01$

[a,b,c] same letters indicate difference is nonsignificant

2.4.11 By-Year Analysis of Copulation Behaviour for Pairs with Copulation Data for All Four Years

A repeated measures ANOVA was conducted on pair and extra-pair copulations, and pair copulation success rates for 13 pairs for which behavioural data were recorded in each of the four study years (see Methods). The results are very similar to those presented for the larger data set when data were treated as independent across years (i.e., Sections 2.4.8 & 2.4.9). Specifically, rates/pair/hour of unsuccessful pair copulation attempts differed significantly across years ($F_{(3,36)} = 26.3$, $p<0.01$; univariate test in repeated measures ANOVA), with 1999 having lower unsuccessful PC attempt rates than all other years except 2000 (1999 vs. 1997, $p<0.01$; 1999 vs. 1998, $p<0.01$; Bonferroni adjustment for multiple comparisons), and 1998 having higher rates than all other years (1998 vs. 1997, $p<0.01$; 1998 vs. 1999, $p<0.01$; 1998 vs. 2000, $p<0.01$; Bonferroni adjustment). As well, as seen in the larger data set, there were no significant differences in either the EP successes or unsuccessful attempts per male or per female for these 13 pairs across years. However, hourly rates of successful PCs/pair were not higher in 1998 than in 1999 for these pairs, as was the case for the larger group.

Analysis of the proportion of PC attempts that were successful (PC successes/(PC successes + PC attempts)) over years used only 7/13 pairs, as there were no PC attempts or PC successes observed for six pairs in the Peak period of 1999. Again, as reported in Section 2.4.9, pair success was proportionately higher in 1999 (93 ±11%) than in all other years ($F_{(3,21)} = 12.43$, $p<0.01$, One-way repeated measures ANOVA with arc-sine transformed data; 1999 vs. 1997 (45 ± 30%), $p<0.01$; 1999 vs. 1998 (50 ± 25%), $p<0.01$;

1999 vs. 2000 (64 = 30%), $p<0.03$: Bonferroni adjustment for multiple comparisons applied).

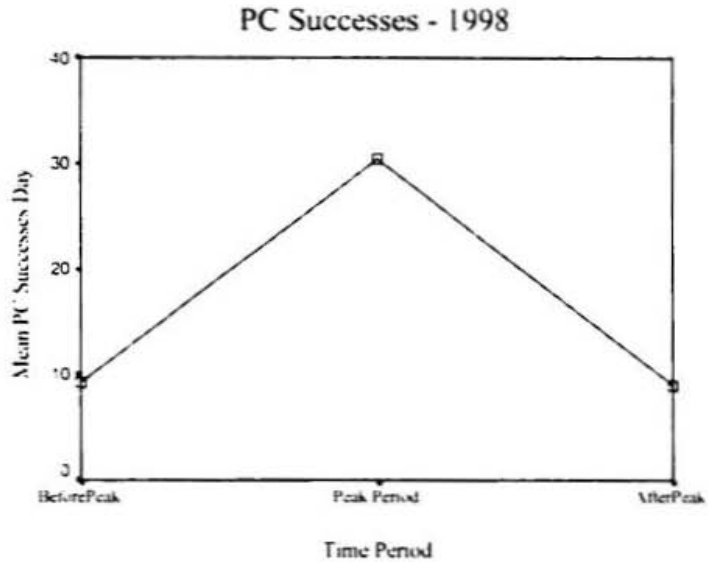

Figure 2-4*a*. Average daily successful PC activity of Common Murres increased from the "Before Peak" period to the Peak period and then declined in the "After Peak" period in 1998.

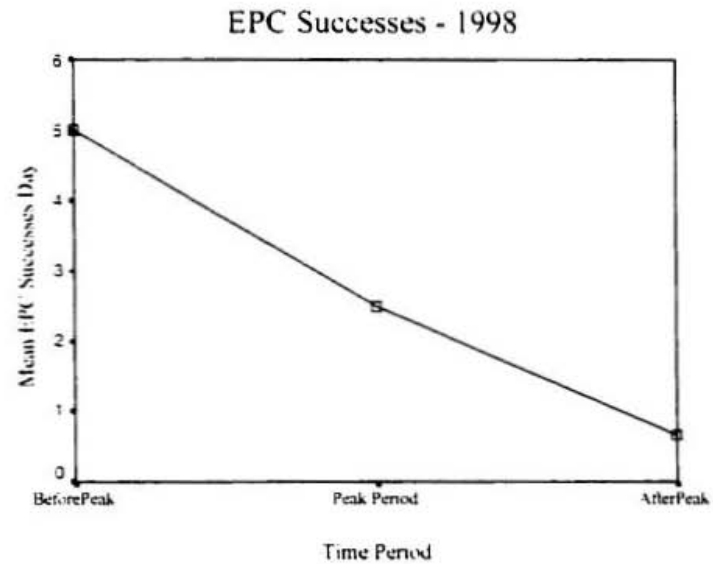

Figure 2-4*b*. Average daily successful EPC activity declined from the "Before Peak" to the "After Peak" period in 1998.

2.4.12 Proportion of Marked Individuals Attempting EPCs in the Peak Period

Across years, the proportions of marked males and females that participated in EPC activity in the Peak period, relative to all marked males and/or females within a given year, was not significantly different (Males: $\chi^2_{(3)} = 3.25$, ns; Females: $\chi^2_{(3)} = 2.50$, ns). The percentage of marked males participating in EPC attempts (successful or not) during the Peak period ranged from 19-36% over the four years, while, for marked females, this percentage was in the range of 21-38%. Of the individual males that made EPC attempts, there was no statistically significant variation in the number of males that were successful in achieving cloacal contact on at least one occasion across years (1997: 3/10 (30%); 1998: 7/12 (58%); 1999: 2/6 (33%); 2000: 6/13 (46%)). Similarly, the proportion of females that accepted at least one EPC attempt (i.e., cloacal contact was achieved) in the Peak period did not vary much across years (1997: 5/6 (83%); 1998: 5/12 (42%); 1999: 4/12 (33%); 2000: 3/11 (27%)).

Overall, both within and outside of the Peak period, 16 different males and 16 different females had successful EPCs at least once during this study. Of these males and females, only five (of 16) males and three (of 16) females had successful EPCs in more than one year. Less than half of all males (11/26) that attempted EPCs in at least one year made EPC attempts in more than one year. Although there were consistent EPC attempts made across years by at least five males, it appears that for most individuals performing EPCs was not a persistent behavioural strategy across years (Section 2.4.22).

2.4.13 Initiation of Copulation and Copulation Type

It was possible to determine who initiated copulation behaviour in the Peak periods of 1997-2000 inclusive for almost half of all attempted and successful copulations (446/943 copulations = 47%; excluding multi-male EPC attempts). When initiation was determined, it was categorized as male-initiated, female-initiated, or initiated by both male and female. There was a significant effect of year on the proportion of copulation initiations that were categorized as determined vs. undetermined (proportion of *undetermined* initiations: 1997: 66%, 1998: 52%, 1999: 31%, 2000: 46%, $\chi^2_{(3)} = 36.9$, $p<0.01$). This may reflect an improvement over time in the ability of some observers (those who were at the site for more than one year) to determine which sex initiated copulation activity.

Overall, males initiated 51% of copulations, females initiated 30%, and 19% were initiated by both partners. Examining the copulations by type revealed that there was a significant relationship between type of copulation and which sex initiated it ($\chi^2_{(2)} = 55.3$, $p<0.01$; Table 2-6). Specifically, males initiated 82% of EPCs (successes and attempts combined), while females initiated 14%, and only 4% were initiated by both partners. For PCs (successes and attempts combined), males initiated only slightly more copulations than females (41 vs. 35%), while 24% of these copulations were initiated by both.

Table 2-6. Chi-square analysis of the independence of copulation initiator and copulation type for all years combined (n= 446 cases).

Copulation Type	Initiator		
	Male	**Female**	**Both**
(Attempts + Successes)	Obs (Exp)	Obs (Exp)	Obs (Exp)
EPC	87 (54)	15 (31.8)	4 (20.2)
PC	140 (173)	119 (102.2)	81 (64.8)

$\chi^2_{(2)} = 55.3$, $p<0.01$

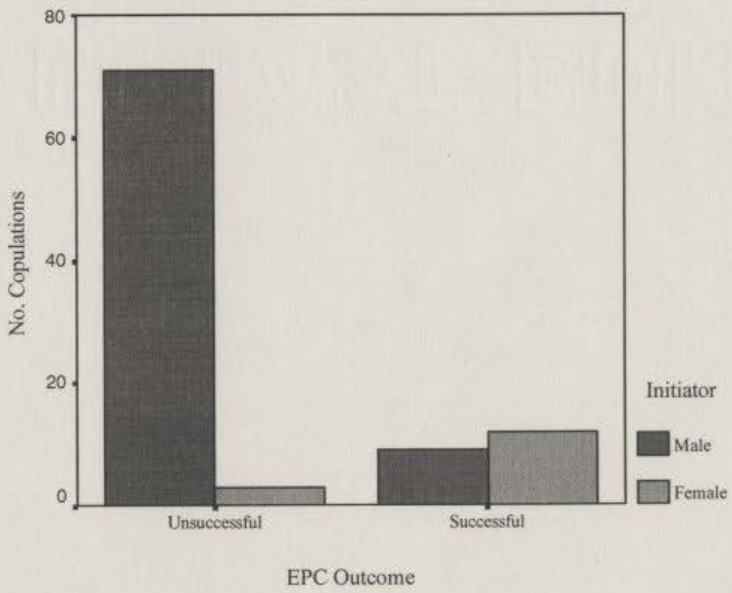

Figure 2-5. Success of copulation in Common Murres is related to the sex of the initiator of the copulation attempt.

2.4.14 Initiation of Extra-Pair Copulations and Outcome

Whether or not an EPC is successful is likely related to who initiates the event. In order to determine whether copulation initiator and outcome were independent of each other for EPCs, the factors Initiator (Male, Female) and Success (Yes, No) were cross-tabulated. This analysis (n = 95 EPCs) showed that outcome was related to initiator ($\chi^2_{(1)}$ = 30.8, $p<0.01$, Yate's correction applied; Table 2-7*a*; Figure 2-5). Males initiated more and females fewer unsuccessful EPCs than expected. Most male-initiated EPCs were unsuccessful, while most female-initiated EPCs did result in cloacal contact. In contrast, male-initiated PCs were proportionately more likely than male-initiated EPCs to be successful (Table 2-7*b*). As well, female-initiated PCs were proportionately more successful than male-initiated ones ($\chi^2_{(1)}$ = 11.73, $p<0.01$; Table 2-7*b*). In total, there were 436 943 copulations that resulted in cloacal contact; 45 (10.6%) were EPCs.

Table 2-7*a*. Chi-square analysis of the relationship between the gender which initiated EPC and the copulation outcome, collapsed across years (n= 95 cases).

	Initiator	
Copulation	**Male**	**Female**
Success (EPC)	Obs (Exp)	Obs(Exp)
NO	71 (62.3)	3 (11.7)
YES	9 (17.7)	12 (3.3)

$\chi^2_{(1)}$=30.8, p<0.01, Yate's correction applied.

Table 2-7*b*. Chi-square analysis of the relationship between the gender which initiated PCs and the copulation outcome, collapsed across years (n= 238 cases).

	Initiator	
Copulation	**Male**	**Female**
Success (PC)	Obs (Exp)	Obs(Exp)
NO	65 (52)	34 (47)
YES	60 (73)	79 (66)

$\chi^2_{(1)}$=11.73, p<0.01

Interestingly, over half (8/15) of the female-initiated EPCs were made by two females (22F and 84F), both of whom experienced divorce. For 22F, female-initiated EPCs were seen both in 1997 (when she was still paired with her mate from 1996) and in 1999, which was the year she was divorced from her mate of 1998 (who was different from the 1997 mate). Female 84 (84F) was observed *soliciting* EPCs only in the year following divorce, which occurred when her mate left their site and paired with another female. Each of these EPCs resulted in cloacal contact between the male and female. For the majority of these EPCs (6/8), the female mates of the solicited males were absent when the EPCs occurred. Four (of the 15) female-initiated EPCs involved (presumably) different unmarked females, who all solicited a single known male, in 1998. This male was unpaired at the time of the EPCs, but became the mate of 22F later that season. For 2 3 remaining cases of female-initiated EPCs, which occurred consecutively and involved the same individuals (3F, 9M), the male and female mates of the participants were absent from the plot when the copulations occurred. This female subsequently divorced her mate later this same year (1998), but the male (9M) remained with his mate. The attendance status of the mates for the participants (4M, 16F) in the final case of female-initiated EPC was unknown, but the pair remained with their respective mates.

2.4.15 Female Site Attendance is Related to EPC Successes

A group of 28 females were selected to assess 1) which factors might be related to EPC activity by females and 2) whether pair bond stability, determined by whether a pair divorced in any year, was related to female and male EPC behaviour. Rationale for

selecting these 28 cases were: 1) there were consistent behavioural and site attendance data for the majority of these females for each of years 1998-2000, and 2) if there were not data for these females in all years, they were included if they were involved in EPC attempts or successful EPCs in any one of the years, or if the pair experienced a divorce in any year (from 1997-2001). One female (89F), who was replaced at her site by another female in early 1998 and subsequently left the ledge, was not included in these analyses as there are no behavioural data for her from early 1998-2000. Due to small sample sizes of both females that accepted EPCs and divorced pairs, as well as heterogeneity of variances for many behavioural measures of interest, data analyses were performed using nonparametric statistics including the Kruskal-Wallis test and the Mann-Whitney U test (corrected for ties).

With respect to EPC activity, females belonged to 1 of 3 groups: 1) females that took part in successful EPCs, i.e., they accepted at least one EPC attempt, 2) females that experienced EPC attempts by males but did not accept them, and 3) females that did not experience EPC attempts by males. For each year separately, females in these groups were examined to determine if they differed on any of the following measures: 1) the average proportion of attendance spot checks for which each female was present in the forenoon of the Peak period (arc sine transformed), 2) the average proportion of attendance spot checks for which each female's mate was present in the forenoon of the Peak period (arc sine transformed), 3) the total number of EPC activities (attempts + successes) of each female's mate, and 4) the PC success rate (number of PC successes/ PC attempts + PC successes) for the Peak period for each pair (arc sine transformed).

In 1999, females which accepted EPC attempts (n = 4) attended the ledge significantly more (Median: 22.2%; Range: 32.9%) than females which did not have any successful EPCs (Median: 4.9%; Range: 21.4%; n = 20 (Groups 2 & 3 combined); z = -2.11, p<0.04; Mann-Whitney test corrected for ties). A similar trend was seen in 2000, with females that performed successful EPCs (n = 3) spending more time at the site than other females (n = 21; Median (range): 34.0% (47.2%) vs. 24.5% (22.7%); z = -1.86, p<0.06). Neither mate attendance, mate EPC activity, nor PC success rate differed among these groups of females.

Interestingly, male attendance was positively correlated with the number of EPC attempts made in 2000 ($r_{(22)} = 0.56$, $p<0.01$), and marginally correlated with the number of EPC successes per male ($r_{(22)} = 0.42$, $p = 0.053$). In other years, there was a trend towards a significant relationship between male attendance and EPC attempts (1998: $r_{(25)} = 0.39$, $p = 0.055$; 1999: $r_{(24)} = 0.40$, $p = 0.056$), but no relationship between a male's attendance and the number of successful EPCs obtained (1998: $r_{(25)} = 0.20$, $p = 0.34$; 1999: $r_{(24)} = 0.21$, $p = 0.34$).

2.4.16 Stability of the Pair Bond and EP Copulations

It became apparent that the stability of the pair bond over the years of this study was a factor likely related to the performance of EP copulations, especially by females. From 1997-2001, there were 7 cases in which pairs divorced, indicating (by definition)

that an unstable pair bond existed between the male and female. Six (of 28) of these females were included in the analyses (the seventh female (89F) is excluded as she left the ledge in 1998). Once a female was labelled as unstable due to a divorce, this label remained for both previous and subsequent years, regardless if the female initiated or was the victim of the divorce, or if she subsequently re-mated (see Methods).

To investigate the effect of pair bond stability on the EPC activity of females, the 28 females were classified as either having stable or unstable pair bonds. For each of the years 1998, 1999, and 2000, the number of EPC attempts for each female was compared to the mean number of EPC attempts/female, and a difference score was calculated for each female. An analogous procedure was used to calculate a difference score for each female in each year for EPC successes. Each female's EPC success rate (EPC success/(EPC success + EPC attempts)) was also compared for those females that had experienced at least one EPC attempt (whether accepted or not). Analyses were performed on the difference scores for stable vs. unstable female's EPC attempts and EPC successes, and for EPC success rates. The median value of the difference score for each group (stable vs. unstable) and the range of the scores are reported. A negative median difference score value would indicate that the females had fewer EPCs than average, while a positive value would indicate that they had more EPCs than average for that year.

In 1998 and 1999, females with unstable pair bonds participated in more successful EPCs than females in stable pair bonds (1998: $z = -2.61$, $p<0.01$; 1999: $z = -$

2.57, p<0.01; Table 2-8). In 2000, this difference between EPC successes of stable and unstable females was not significant (z = -1.86, ns).

Table 2-8. The median difference score (and range) for EPC successes by females that experienced stable vs. unstable pair bonds (1998, n=26; 1999, n=24; 2000, n=24). To obtain the difference score, the number of successful EPCs for each female was subtracted from the mean number of successful EPCs for all females in each year separately. A positive median score indicates that the females in this group experienced more successful EPCs than average.

	1998*		1999*		2000	
	Median	Range	Median	Range	Median	Range
Stable	-0.31 (n=21)	1.0	-0.29 (n=18)	1.0	-0.71 (n=18)	1.0
Unstable	0.69 (n=5)	4.0	0.21 (n=6)	3.0	-0.71 (n=6)	15.0

*p<0.05.

In 1999 and 2000, females from unstable pair bonds also had a higher percentage of successful EPCs/(total EPC attempts - EPC successes) than females from stable pair bonds (1999: z = -2.32, p<0.03; 2000: z = -2.10; p<0.04; Table 2-9). There was no difference in EPC success rates for 1998.

Table 2-9. Median EPC Success rates (difference scores) for females from stable vs. unstable pair bonds (1998, n = 14; 1999, n = 12; 2000, n = 12). Only females experiencing at least one EPC attempt were included. A positive median score indicates that females in that group experienced a higher EPC success rate than average.

	1998		1999*		2000*	
	Median	Range	Median	Range	Median	Range
Stable	0 (n=10)	1.0	0 (n=8)	0.25	0 (n=8)	1.0
Unstable	0.27 (n=4)	0.50	0.55 (n=4)	0.67	0.83 (n=2)	0.35

*p<0.05

There was no difference in the number of EPC attempts made by males on females from stable pairs compared to females from unstable pairs.

2.4.17 Pair Stability and Mate Behaviour

There was no apparent relationship between pair bond stability (i.e., the category assigned to the female) and the behaviour of the female's mate. Total male EPC activity did not differ significantly between groups, nor did male attendance for any year. It is

interesting to note that the five male "repeat copulators" (i.e. males which performed successful EPCs in two or more years; Section 2.4.22) were in pair bonds categorized as stable.

2.4.18 Pair Bond Stability and Reproductive Parameters

Not surprisingly, pair stability was significantly related to the proportion of chicks hatched per female from 1997-1999. These were the years immediately *preceding* the years for which the current behavioural analysis was conducted (1998-2000) and were analyzed since reproductive parameters (hatching success, parental performance) in one year might influence a mate's EPC behaviour in the following year, but cannot in the concurrent year. Significantly more chicks were produced over the three years for females from stable bonds than for females involved in unstable pairs (Medians: Stable (n = 20): 100% (range 0-100%); Unstable (n = 6): 67% (range 0-67%); $z = -2.30$, $p<0.03$, Mann-Whitney test). In several cases, females from unstable pairs failed to lay eggs either before and/or after their divorce, and/or their eggs failed to hatch, and/or their chick failed to fledge. As well, there were four cases from 1997-2001 in which first egg loss occurred (due to predation or unknown causes) and, as a result, no chick was produced at that site for the year of the loss. This contrasts with most cases of first egg loss where a successful second egg is typically laid. Three of these four cases involved unstable pairs, and the remaining case involved a pair (pair 6), for which extra-pair paternity (EPP) of a chick was detected (see Section 2.4.20). Pair bond instability was also related to two other cases of EPP.

Since pair bond stability might be related to the quality of the individuals in the pair (i.e., females may be more likely to remain in a stable bond with a high quality male: Ens et al. 1993), and individuals in stable relationships are more likely to produce young successfully, an index of male parental effort (and, arguably, an index of male quality) was assigned to each male that helped raise at least one chick from 1997-1999. To develop this index, the mean number of fish per day that the male brought to its chick was summarized for three chick age periods: Days 1-4, Days 5-8, and Days 9-12. The average fish delivery rate per male for each period was then compared to the overall average fish delivery rate for all males under examination, and each male was ranked as "above average", "average" or "below average" for each chick age period. A yearly ranking was then assigned, based on the majority of ranks given to the male (e.g., a male was "above average" if he had 2 3 "above average" ranks).

For each of 1998, 1999, and 2000, mate fish delivery performance in the prior year and concurrent year was unrelated to the performance of successful EPCs by females. As well, pair stability (Stable vs. Unstable) and the ranking of male parental effort (Average or Above Average vs. Below Average) were independent, indicating that neither EPC successes nor pair bond stability were related to mean male ranking for fish delivery rates to chicks (All χ^2 analysis nonsignificant; data not reported).

2.4.19 EPCs and Chick Paternity

During the Peak periods of 1997-2000, 12/45 different females (27% of all females observed) accepted at least one EPC attempt from a male. An additional four females participated in at least one EPC success outside the Peak periods of 1997-1999, for a total of 16 (36% of known females) females with successful EPCs. For 12 of the 16 females that accepted EPCs, genetic testing was conducted to determine paternity of at least one of their chicks (Pairs 5, 6, 7, 11, 12, 17, 21, 22, 23, 24, 84, and 93; Chapter 3). For the remaining cases, either chicks were not produced, or chicks and/or adults of the family could not be caught. DNA analysis was conducted only on families in which all members were sampled. Three of these females (6F, 84F, 93F) had chicks sired by extra-pair males in 1998, while the chicks of the remaining females had DNA profiles (genotypes) consistent with paternity by the female's social mate.

Copulation behaviour was not recorded in the year of the occurrences of extra-pair paternity (EPP) for one of the females with an EPP chick (93F), because the pair was not marked in the pre-laying period of this year. A salient feature of the behaviour of the other two females (6F, 84F) with extra-pair chicks in 1998 was the observation that, although these females were not seen to have had successful EPCs in this year, they did have successful EPCs in other years, *and they were not seen to have accepted any PC attempts by their mates at any time in 1998*. This was in spite of five PC attempts made by 6M and two PC attempts made by 84M in this year. This contrasts starkly with the behaviour of other paired females which participated in successful EPCs (with the

exceptions of 23F in 1997, 21/89F in 1998, and 93F in 1999, see below); for the majority of these females (8/12), their successful EPCs were followed by a minimum of one (Mean = SD: 4.1 = 1.6 PCs) successful PC.

The detailed case studies of the females who were observed participating in EPC successes follows. The 12 individuals for which at least one chick was subjected to paternity testing are examined first. Notes from the 2001 breeding season, which were not analyzed in this paper, are nonetheless included for these females when considered relevant.

1) **6F**: Pair 6 successfully hatched a chick in 1996, 1997 and 1998. In both 1996 and 1997 the chicks' DNA profiles were consistent with that of 6M being the father. 6F did not participate in any observed successful EPCs during the entire 1997 and 1998 pre-laying season, nor during the Peak period of 2000. In 1998, the year that the chick was determined to be of extra-pair paternity, 6F was subjected to two EPC attempts (by 5M) that were rejected by her. As already stated, there were no successful PCs observed at all in 1998, despite five PC attempts by 6M. On one day (May 25, 1998) following the Peak period, 6F was observed alone at the site (although no EPCs were observed). The pair was not observed to be in attendance together on subsequent observation days in the pre-laying period. In 1999, 6F performed one successful EPC, the day after she and her mate had been at their site together for the first time that year. After this and prior to her egg-laying, a successful PC was observed. In 1999, her egg did not hatch in spite of incubation and, thus, was either infertile, or the chick embryo non-viable. In the

following year, 2000, only PCs were observed and an egg was produced but was lost due to unknown causes. The exact date of egg loss was undetermined, but the egg was incubated for more than the normal 33 day period without hatching, so was presumed to be either infertile or non-viable. In 2001, 6F participated in a successful EPC, six days prior to her egg laying date, which was followed two days later by a successful PC. Once again, her egg failed to hatch.

2) **84F**: This female and her mate was not banded until 1998. However, it is possible that pair 84 was the pair at the "old s9" site in the pre-laying period of 1997 (see case number 16). 84F successfully produced chicks in 1997 and 1998. The 1998 chick was determined to be of extra-pair paternity. In 1997, there were no behavioural observations made for pair 84 during pre-laying (unless this pair is actually "old s9"), so copulation activity was unknown for that year. In 1998, the pair was not seen to have engaged in any successful PCs at any time, despite two PC attempts made by 84M. However, the female was not seen to have taken part in any EPCs in this year either, although the EPP of her chick indicates that she did so. Following the last recorded unsuccessful PC attempt by this pair, the female was not seen at the ledge on the subsequent four observations days of the pre-laying period. In 1999, 84M left the site and paired with an unmarked female (no egg was produced by 84F in 1999). In 2000, 84F remained unmated and took part in 15 successful EPCs during the Peak period. In 2001, 84F paired with a new mate and both first and second eggs were laid, but these both disappeared from the site.

3) **93/95F**: No behavioural data were recorded for this pair in the pre-laying period of 1998, as they were first banded during chick-rearing in that year. DNA analysis determined that the chick was of extra-pair paternity in 1998. In early 1999, 93F was seen performing a successful EPC with an unbanded male (possibly 95M) on the same day that she and 93M were reunited for the season (the EPC occurred after a successful PC). No further successful PCs occurred after the EPC, and, 11 days later, the female left 93M and moved to the site of a neighbouring, unpaired male (95M), with whom she remained paired in 2000 and 2001. No further EPCs were observed for this female in 1999 or 2000. In 1999, no chick was produced by 93/95F, but a chick was successfully hatched in 2000 and 2001 by the new pair.

4) **21/89F**: In 1996 and 1997, 21F and her mate produced a chick in each year. In 1996, paternity analyses indicated that the chick was sired by 21M. In 1997, paternity analysis could not be carried out due to failure in obtaining DNA from this chick's feather sample. In 1998, the pair divorced, likely as a direct result of fighting between 21M and a Razorbill (*Alca torda*) living on the ledge (Walsh et al. submitted). Successful EPCs were recorded for 21/89F on five different days in 1998 (the year of divorce from 21M). EPCs occurred one day prior to the first time both 21F and 21M were together at their site, i.e., 21M had not yet arrived in the ledge. 21F's performance of successful EPCs on the remaining four days were probably due to the low attendance of 21M at the site, as he was continuously chased away by the Razorbill. Three of the five EPCs performed by 21F after her

reunion with 21M were with 89M, with whom she paired later in 1998. This new pair successfully produced a chick late in the season. In 1999, 21/89F performed EPCs on two different days prior to being together at the site with 89M, and again following their reunion. No EPC successes were recorded for 21/89F in 2000. In 1998, a successful EPC with 89M (her future partner) was followed within the hour by an EPC success with an unbanded male. As the 1998 chick was not caught, its paternity is unknown. There were, however, successful PCs recorded in each year, including successful PCs in 1998 with 21M. Chicks were successfully produced in 1999 and 2000 (but were not caught). In 2001, their egg was lost and 21/89F was frequently seen at the site alone, while 89M visited the site of a nearby female whose chick had recently fledged.

5) **23F**: Data was obtained for this pair in 1996 and 1997 only. In 1996, the paternity of the chick matched 23M. In 1997, 23F performed several successful EPCs with 29M on May 24, well after she had been reunited with 23M on May 17, 1997. 23F was not seen to have engaged in PCs with her mate on any of the eight subsequent observation days in the pre-laying period. An egg was produced at this site, but was preyed on by a gull and the female did not re-lay. In 1998, this pair moved to the ledge just south of study site, and their behaviour could no longer be observed.

6) **24F**: This female was observed to have had one successful EPC in 1998 only, which occurred during the day, but just prior to the time, that pair 24 was first reunited for the season. At least four successful PCs followed on the same day.

This pair produced a chick in each of the years from 1996 to 2001. Genetic analysis on chicks from 1996 and 1998 (the only years in which the chicks were caught) indicated that 24M was the genetic father.

7) **22F**: This female was seen to have successful EPCs in each year of the behavioural study (1997-2000). In 1996, an egg was laid by 22F but was subsequently lost. A chick was successfully hatched in 1997 by the pair. In 1998, 22M did not return, and the female paired with a new male. The egg produced in 1998 did not hatch despite incubation, so might have been infertile, or the embryo non-viable. This pair subsequently divorced in 1999, and 22F paired with a new male and successfully produced a chick. The paternity analysis of both chicks (1997 and 1999) showed that the mate in each year was the most likely father of the chick for that year. In each of years 1997 and 1999, there were three successful EPCs performed by 22F (following the first time that she & her mate of the previous year were together). These EPCs were subsequently followed by four and six successsful PCs, respectively. In 2000, 22F's mate from 1999 did not return to the ledge and was presumed dead. She was observed performing EPCs on the first day of behavioral observations only (May 15). 22F subsequently took another new mate in 2000 and produced a chick. This mate did return in 2001, and a chick was again successfully produced. However, the paternity of these latter chicks has not yet been analyzed.

8) **12F**: Pair 12 was unknown in 1996, even though the male had been banded in 1986 by D. Cairns (either the male was not at the site in 1996, or he went unnoticed). No egg was laid at site 12 in 1997, although it is uncertain whether the female of that year was the same as in 1998, as 12F was not banded until 1998. A chick was produced successfully in 1998 only and paternity analysis determined that 12M was the sire. 12F was not seen to have participated in any EPCs in this year. In 1999, this female took part in one successful EPC, occurring both after the pair had been seen together in the ledge for the first time and in the male's presence, which was followed by three successful PCs. However, the egg produced in 1999 did not hatch and the egg produced in 2000 was lost due to unknown reasons. In 2001, 12M left the site and paired with a female (10F) whose mate did not return (this pair successfully hatched a chick). 12F paired with a new (unmarked) male and produced an egg, but the egg got wedged under a rock and could not be incubated, so it did not hatch.

9) **11F**: Chicks were hatched successfully by this pair in each year from 1997-2000 (the pair was unknown in 1996). DNA analyses on the chicks from 1997 and 1999 were inconclusive, as the chicks did not amplify at two loci (see Chapter 3). However, there was no evidence of EPP from the loci which did amplify. In 1999, 11F participated in one successful EPC, on the day following her reunion with 11M. There were two PC successes performed 5 days following the female's EPC success, after the Peak period in 1999. In 2000, there were no EPC successes

recorded for this female. 11F laid an egg in 2001, but it was unfortunately lost due to experimenter-induced activity.

10) **5F**: Chicks were produced successfully from 1997-2001 at this site (the pair was unknown in 1996). The chicks produced in 1997, 1998, and 1999 were all determined to be fathered by 5M (2000 and 2001 chicks were not analyzed). 5F was seen to have accepted two EPC attempts in 1999 only; this was the year in which she was not reunited with her mate until nine days after her arrival on the ledge. The successful EPCs occurred on her first day on the ledge in 1999; one was with 27M, while the other was with an unmarked individual. Upon their reunion in 1999, pair 5 had at least five successful PCs.

11) **7F**: This pair successfully produced chicks from 1996-2001. Chick paternity testing from 1996-1999 was conducted, but was inconclusive as the DNA was not retrieved from the 1996, 1997 and 1998 chicks and the 1999 chick failed to amplify at two loci (see Chapter 3). At the loci which did amplify for the 1999 chick, no evidence of EPP was detected. 7F had one successful EPC recorded in 1998, which occurred prior to, but on same day as, her reunion with 7M. It was followed by six successful PCs in the pre-laying season of that year.

12) **17F**: In 1998, 17M mate did not return to the ledge and was presumed dead. 17F had her only successful EPC in that year with an unbanded male on the first day of behavioural observations. A chick had been produced in the previous year, and its

paternity matched 17M from 1997. In 1998, 17F took a new mate and successfully produced a chick, whose paternity matched the new male. The EPC in 1998 was followed by twelve PC successes with the female's new mate. Again in 1999, a chick whose paternity matched the new 17M was produced. Chicks were also hatched in 2000 and 2001.

There was no analysis of paternity for any of the chicks produced by the following females:

13) **3/83F:** In 1997, pair 3 successfully hatched a chick, but the chick was subsequently lost to predation. In 1998, 3F left the site and paired with 83M (whose mate failed to return that year). 3F was seen to have only one successful EPC in all of 1998 during which she solicited 9M. This EPC occurred after her first reunion with her old mate and was followed by three successful PCs several days later (with the old mate, 3M), but prior to their divorce. No EPCs were observed between 3/83F and 83M in 1998 prior to the female's divorce, nor were there any PCs afterwards. An egg was laid at site 83 by 3/83F in 1998, but was lost. In 1999 and 2000, there was a chick successfully produced at the 83 site by the female, but neither successful PCs or EPCs were observed in these years. In 1999, the pair arrived late in the pre-laying season and the female was not observed on the ledge at all prior to the date of her egg-laying. A chick was successfully produced at this site in 2001.

14) **25F**: Eggs were laid and chicks successfully produced at this site from 1996–2001 inclusive. In each of 1997 and 1998, 25F participated in one successful EPC during the Peak period with males from nearby sites (23M in 1997; 24M in 1998). In both years, these EPCs occurred after the pair had been first reunited for the season and successful PCs (three in 1997 and four in 1998) followed the EPCs several days later. No EPCs were performed by this female at all in 1999 or in the Peak period of 2000. In 1999, three PC successes were observed following the Peak period.

15) **9F**: This pair was unknown in 1996, but 9F accepted one EPC attempt in 1997 (after she had been reunited with 9M) which was followed by five PC successes 12 days later. Eggs were laid and chicks hatched in each year from 1997-2001. No more EPC successes were recorded for this female.

16) "**old 9F**": This pair was observed in 1996 and 1997. In 1997, the female had only one successful EPC (the day following her reunion with her mate) followed by seven successful PCs in subsequent days. An egg was produced in this year, but it is uncertain if it hatched. This could be due to the fact that, by chick rearing, this site had been renamed "s84". Thus, it is possible that this "old s9" pair (a bridled male and an unbridled female) was pair 84 (see case number 2 for the case history of 84F). If this was the case, the number of females participating in EPC successes during the Peak periods of 1997-2000 is reduced from 12 to 11, as "old s9F" and "84F" could be the same individual.

2.4.20 Summary of Case Studies

In general, the previous case studies of females which have been observed accepting at least one EPC attempt can be summarized as follows:

1) Extra-pair fertilizations are unlikely if females have successful PCs after successful EPCs. Most successful EPCs performed by females were followed in the pre-laying season by successful, and proportionately more, PCs. The chicks of seven females who performed EPCs which were followed by PCs had no evidence of EPP. There were nine pairs from 1997-2000 in which no PCs were observed (whether PC attempts were made by the male or not); in three of these cases, the pairs involved produced an EPP chick in 1998 while, in the remaining cases, no (or inadequate) genetic information on the chicks was available and paternity could not be determined.

2) Females' successful EPCs do not typically occur prior to their first reunion with mates in a season. For most females, successful EPCs occurred after the females had already reunited for the season with their mate. There were four instances in which a female's EPC success occurred prior to her mate arriving in the ledge for the season: 5F, 7F, 17F, and 24F. Each of these females performed successful EPCs on a single (but different) day (5F performed two EPCs on one day, while 7F, 17F, and 24F participated in only one successful EPC each). In the case of 17F, the EPC occurred in the year which her mate did not return to the ledge, and was presumed dead. The pair bonds of these four females were catergorized as stable and these females were all highly productive, producing

chicks successfully in all years for which there are data on them. As well, the majority of chicks belonging to each female were analyzed for paternity; all of these chicks had paternity consistent with that of the female's mate.

3) There is a high incidence of divorce among females which accepted or solicited EPC attempts. Ten (of 15 = 66.7%) female-solicited EPCs involved females who were or who became divorced. As well, 6 (of 16 = 37.5%) females that accepted at least one EPC during the study were divorced. It is worth noting that there were only seven divorces from 1997-2001 and all but one female (89F, who left the ledge) were involved in EPCs. For five of these females (and possibly all, if 84F and "old s9"F are the same individual), at least one successful EPC per female was recorded in the year of or the years before the divorce. As well, there is only one case (3 83F) for which there were no EPCs recorded following the female's divorce. Four other females participated in EPCs in the year of or the years following their divorces, but the circumstances under which these occurred varied considerably:

a) 84F remained unpaired for the year of and the year after her divorce and performed many EPCs;

b) 93 95F performed EPCs in the year of her divorce, but was not seen to do so in later years, presumably after she had established a pair bond with 95M;

c) 21 89F performed EPCs in the year of and the first year following her divorce and pairing with 89M;

d) 22F had EPCs in each year, as she had a different mate in 1997, 1998, 1999, and 2000. A divorce occurred in only one year (in 1999 with mate from 1998). New mates were taken in 1998 and 2000 due to the non-return of the male from the previous years.

The divorce of the final female (12F) occurred in 2001. This female was known to have had an EPC (witnessed by her mate) prior to divorce. After divorce, she had copulations with a male that she subsequently paired with and produced an unsuccessful egg with in 2001.

2.4.2 Successful Male Extra-Pair Copulators

Of the 16 males that obtained at least one successful EPC over the entire study, only five obtained successful EPCs in more than one year:

1) **19M**: This male had successful EPCs with an unmarked female in 1997 and a (presumably different) unmarked female in 2000. It is unknown whether chicks were subsequently produced in any year of this study at his site, as behavioural observations were not possible due to its poor visibility from the blind.

2) **85M**: 85M had one EPC success with 21/89F in 1999 and four successful EPCs with the unpaired 84F in 2000. There was a chick successfully produced at this male's site each year from 1997-2001.

3) **20M**: In 1998, 20M had four successful EPCs with 21/89F (this was the year during which this female divorced her mate). In 2000, 20M had four successful EPCs with the

unpaired 84F, two of which the female initiated. There was a chick produced at the site in each year from 1996-2001.

4) **6M**: In 1997, 6M had a single successful EPC with 22F, which was initiated by her. In 1999, he again had a successful EPC with 22F, as well as a single EPC success with 21 89F. There were chicks at this site from 1996-1998, although the chick from 1998 was not fathered by 6M. There were no chicks hatched at the site from 1999-2001.

5) **25M**: In each of 1998, 1999, and 2000, 25M had successful EPCs with a single female-22F. Three successful EPCs were recorded between these individuals in 1999, of which one was female-initiated. A single EPC occurred in each of the other years. There was a chick produced successfully at this site yearly from 1996-2001.

Thus, for the majority of these males (with the known exception of 6M and the possible exception of 19M), there were chicks successfully produced at their sites in all years. For two males, 6M and 25M, there is a pattern of recurrent EPCs with the same female (22F) in two or more years. Indeed, for 25M, this is the only female with which he ever had successful EPCs, and some of these EPCs were known to have been solicited by the female.

2.5 Discussion

2.5.1 Timing of Copulation Activity

Copulation activity on this Common Murre ledge was higher during the pre-laying period (i.e., the two attendance peaks known as the "Peak period") than it was in the period after the first egg was laid, an expected pattern similar to that reported in Birkhead et al. (1985) and Hatchwell (1988). Hatchwell (1988) reported that PC rates peaked approximately 12 days prior to the median egg-laying date in his study colony, and that the numbers of forced EPCs (i.e., EPCs that the females resisted, of which only 6% were successful) increased during this period compared to earlier. In my study, no EPCs that were resisted by the female were seen to be successful, i.e., resulted in cloacal contact; thus, all resisted EPCs were categorized as unsuccessful EPC attempts. These unsuccessful EPCs showed a similar pattern to that reported in Hatchwell (1988) of increasing from the "Before Peak" to the Peak period, which began, on average, 12 days prior to the first egg being laid on the ledge. It is difficult to determine when the highly successful unforced EPCs occurred in Hatchwell's study. He reported that half (9/18) of these occurred between Day –25 (relative to egg-laying) and the day of egg-laying, but did not indicate when the remaining nine occurred.

Data from the current study suggest a pattern of murre EP activity at Great Island that was different from that observed by Hatchwell (1988). Observations for more than one day prior to the Peak period in this study were obtained for 1998 only. In that year, significantly more successful EPCs per day occurred from 17-19 days prior to the first

egg being laid in the site (i.e., the "Before Peak" period) than after the Peak period, with an intermediate number of successful EPCs/day occurring within the Peak period. This suggests that EPCs which resulted in cloacal contact at this site were more common during the very early period of pre-laying than later. In general, there were slight decreases in both PCs and EPCs from the Peak period of attendance to the "After Peak" period in all years except 1999, the year during which males and females did not show the typical cyclic peaks of presence and absence (Wilhelm et al. 2000). During 1999, the frequency of PC attempts increased from the Peak period to the "After Peak" period; this is likely related to the increased attendance of females at the site following the beginning of egg-laying in 1999 (Figure 2-2).

There is some debate about when the fertile period in female murres begins. While Birkhead (1985) assumed that females were fertile from approximately 12 days before egg-laying, Hatchwell (1988) assumed that this period began earlier (Day –25), based on his observation that one female who did not attend the colony for 17 days subsequently laid a fertile egg. It would be valuable to know the actual length of the Common Murre's fertile period, as EPCs which occur prior to or after this period could have different functions from those occurring within the fertile period (Wagner 1991*a*; Hunter et al. 1993). There is evidence that Common Murres possess sperm-storage tubules (SSTs), in which sperm may be maintained for an unknown duration and are subjected to competition for fertilization of the single ova if the female is inseminated by another male (Birkhead & Del Nevo 1987). While both Birkhead et al. (1985) and Hatchwell (1988) calculated theoretical probabilities for the fertilization success of an EPC based on the

proportion of EPCs to PCs, they suggested that a "last male advantage" in murres is possible, a pattern seen in many species of birds (Birkhead and Hunter 1990; Birkhead & Møller 1998). In this scenario, the last male to inseminate the female would sire the chick eventually produced. While females in the current study may have stored sperm from successful EPCs, it was most common to observe frequent successful PCs in the period following EPCs and prior to egg-laying. In cases when this occurred, no evidence of extra-pair paternity was found (as has been found in Fulmars by Hunter et al. 1992). For two observed females that produced a chick with an extra-pair male, there were no observed PCs at all during that year. Thus, these data cannot be used to distinguish between mechanisms of sperm competition in Common Murres, as they are consistent with both the "last male advantage" and the "proportional sperm representation" hypotheses.

As reported in the Birkhead et al. (1985) and Hatchwell (1988) studies, copulation behaviour of Common Murres on Great Island was not randomly or uniformly distributed throughout the day. Most copulations occurred in the morning in all three studies. There was no apparent time during which EPC successes tended to occur. Rather, such events were opportunistic in that they most often occurred either when the female mate of the copulating male or the mates of both birds were absent from the colony. This is consistent with the possibilities that: 1) if EPCs were detected by mates, there would be a cost in terms of decreased parental investment or risk of divorce, for example, and/or 2) mate presence in the colony is a form of mate guarding by males and/or females to prevent EPCs (Wagner 1992*b*; Birkhead & Møller 1998; *cf.* Section 2.5.2.).

In this study, there were only two females known to perform EPCs while their mates were present in the ledge. Both females did this on different days in the same year (1999). One female, 22F, was slightly unusual in that she had just divorced her previous mate, had retained the breeding site, and her new mate was a young male for which this was his first breeding attempt. The female had two successful EPCs on the same day with the new mate present, after she had already engaged in courtship and copulation with him. The pair produced a chick which was determined to have been sired by the new mate. Interestingly, the male's chick feeding ability from Days 1-12 were rated as "below average", but this relatively poor performance could be due to either age or experience-related factors (Forslund and Pärt 1995) and not reduced paternal investment (Davies et al. 1992; *cf.* Houston 1995; Schwagmeyer et al. 1999). The other female, 12F, experienced a divorce two years later, when her mate left their site for a recently-widowed female. Since their egg didn't hatch in the year of the female's EPC with her mate present, I could not determine whether 12M decreased his parental investment in response to 12F's activity. Although strongly predicted in the early literature, the reduction of paternal care by cuckolded males does not occur as often as expected; it has been suggested that reducing paternal care could also reduce the assessment of a male's attractiveness or quality made by neighbours which, in turn, could reduce his opportunities to obtain either EPCs or a new mate in the future (Morton et al. 1990; Wagner 1992*c*, 1996; Schwagmeyer et al. 1999). Alternatively, low quality (or young) individuals may not be able to invest in either extensive mate guarding or paternal care,

making the relationship between being cuckholded and reducing paternal care appear causal.

Why two female murres would successfully copulate with another male in the presence of their mates is unknown. It is notable that such behaviour has not been reported for Razorbills, as females were never seen to have accepted an EPC while their mates were present (Wagner 1992*d*). It is possible that the costs of the mate's knowledge of the EPCs were low for these females, although, in one instance, the female (12F) was ultimately deserted by her mate. Enquist et al. (1998) suggested that there could be a logic to such a "ménange à trois", if the female's receptivity to other males actually helped secure more attention or assistance from her mate. This possibility receives some support from 12F's situation in that no PCs were observed in 1999 until *after* the female took part in the EPC. In the case of 22F, it is possible that a pair bond was not yet firmly established with her mate of that year when she performed the EPCs, and that they served as mate sampling (Colwell and Oring 1989; Heg et al. 1993). The fact that both of these females performed EPCs in the same year, 1999, the year in which female attendance at the ledge was low, may be significant. If some unknown ecological factor made female attendance more costly in that year compared to other years, then these females might have taken advantage of the EPC advances of other males with little regard to whether their own mate was present. Certainly, it is impossible to analyze the potential costs of detection of an EPC by a mate based on the anecdotal evidence of two case studies.

2.5.2 Copulation Rates Differ Among Years

There were significant differences among years in the pair, but not extra-pair, copulation activity rates observed in the study site, in spite of equating different years for timing with respect to egg laying. While rates of EP activity (successes and attempts) remained relatively stable from 1997-2000 in the Peak attendance period prior to egg laying, there were significant differences in the average number of pair copulation attempts and proportion of successes per pair among years. In absolute terms, unsuccessful PC attempts per pair were higher in 1998 than in all other years, and all PC activity (attempts and successes) per pair was significantly lower in 1999 (the year of asynchronous pair attendance) than in all other years. Similarly, the PC success rate (number of successful PCs vs. all PC successful and unsuccessful attempts) differed in the opposite direction: PCs were proportionately more successful in 1999 than in other years and less successful in 1998 than in 1999 or 2000. It is likely that the high PC success rate in 1999 was related to the very low attendance of females at the site that year (see below). Thus, in this year when females arrived at the ledge, they accepted most, if not all, of their partner's copulation attempts. In fact, it seems plausible that when females did come to the ledge in 1999, they did so to obtain copulations.

It is interesting that there were such high absolute levels of PCs in 1998, accompanied by the lowest PC success or acceptance rate (only 44% of PC attempts/pair were accepted). This suggests that females were only willing to participate in a certain number of pair copulations, and refused any above and beyond this "copulation limit". It

has been proposed that copulation activity is costly for both males and females (Hunter et al. 1993). Costs of copulation could include danger of predation, loss of time that could be devoted to feeding or nest-building, increased chance of disease or parasite transmission and metabolic costs (Hunter et al. 1993). The first two copulation costs seem relatively unlikely for Common Murres, since copulation occurs in the colony, typically on cliff ledges (i.e., inaccessibility and coloniality are, in part, defences against predation; Birkhead 1978), and time lost that might be devoted to foraging, for example, is due to attendance at the ledge and not acceptance of copulations *per se*. Thus, when female murres refused extra PC attempts from their mates in 1998, it could have been due to increased risk of pathogen transfer from copulations, the metabolic costs of copulating, or to some other factor. Wagner (1996) hypothesized that, for Razorbills, female refusal of pair copulations could act as a "testing of the pair bond", by which females could assess the male's parental commitment following egg-laying. Also, a female might assess a male's quality/vigour by these extra PC attempts. It is unclear yet how these hypotheses could be tested in Common Murres, although a more complete investigation of the relationship between parental care and pre-laying copulation behaviour could be informative.

Temporal variation in EPC activity, inferred by variation in EPP rates, within a population of birds has been described for only a few species to date (e.g., Red-winged Blackbirds, Weatherhead et al. 1994, Weatherhead, pers. comm. cited in Petrie & Kempeaers 1998; Great Tits (*Parus major*), Lubjuhn et al. 1999) and is not well understood. Such variation may indicate that EPC behaviour by males and/or females was

altered in certain breeding seasons, or under particular ecological conditions. Such variation could also indicate that individual females did not alter their EPC rates, but rather, changed their *pair* copulation acceptance rate, perhaps, especially their acceptance for PCs that followed EPCs. As well, variation in EPP may have little to do with temporal changes in overt copulation behaviour of the population, but everything to do with circumstances under which EPCs turn into EPFs. Theoretically, in some species, EPFs may be influenced by post-copulatory, pre-fertilization female control (but see Birkhead and Møller 1993). Our current inability to adequately explain such differences in EPP rates is likely due to the fact that long-term studies of EPCs and EPP are not common: most studies involve one or two field seasons (see Chapter 3), even though the importance of evaluating life-time reproductive strategies, especially in long-lived species such as seabirds, is generally recognized (Stearns 1992). In this study, I can only speculate as to the possible reasons for the high levels of unsuccessful pair copulation activity, along with no difference in the overall *number* of PCs/pair that were accepted in 1998 (at least, compared with 1997 and 2000). Notably, this year, 1998, was also the only year in which EPP was detected.

An analysis of the variation in pre-laying attendance patterns of Common Murres on Great Island showed that the overall attendance of females, and thus, pairs, was highest in 1998 and lowest in 1999 (Wilhelm and Storey, in prep.; Figure 2-2). Compared to 1999, females were at the ledge 3.3 times more in 1998, and 2.4 times more in 2000. As well, pairs were at the ledge together 5.1 times more in 1998 than 1999, and 3.5 times more in 2000 than 1999 (Wilhelm and Storey, in prep.). Thus, in 1998, pairs spent the

most time together on the ledge. It is not surprising, then, that 1998 was also the year of the most pair copulation activity, coupled with the most female refusals of PC attempts. What still remains a puzzle is *why* female attendance was so high in 1998, or, more appropriately, why it was so low in 1999. Weather (temperature, wind speed, and precipitation) effects were investigated and did not differ significantly among years (Wilhelm and Storey, submitted). One obvious explanation could be variation in food availability during the pre-laying season across years. If the food supply (i.e., fish shoals) was farther away from the colony in 1999 than was usual during this period, it might be expected that normal levels of attendance would be too energetically costly for females that may be constrained by the impending metabolic requirements of egg production (Birkhead and Del Nevo 1987) . Wilhelm et al.'s (in prep.) analysis of chick body condition showed no significant decline in 1999 compared to other years, suggesting that by late June- early July, food availability was probably at normal levels. Thus, it is still unclear what factors caused the variation in female attendance, and, hence, the variation in copulation activity over years.

2.5.3 Do Common Murres Mate-Guard?

Mate-guarding has been defined as 1) male behaviour that increases the probability of achieving high certainty of paternity (Birkhead & Møller 1992), and 2) female behaviours that defend the pair-bond from instability (i.e., prevent mate loss to another female) by preventing males from copulating with others (Wagner 1992*b*; Petrie & Hunter, 1993). Presumably, the mate-guarding behaviour by males could prevent the

female from "being enticed" to leave the pair bond, although there are no direct data of which I am aware to support this notion. In colonial species, high nesting density increases the availability of extra-pair individuals with whom paired birds may mate. Also, because foraging grounds are often considerable distances away from the colony (Cairns et al. 1990), individuals are frequently left alone while their mate is away. These facts make mate-guarding in colonial species difficult, and may have selected for the evolution of frequent pair copulation, versus the constant following and physical intervention behaviour seen in other species (e.g., Komdeur et al. 1999), as a method of paternity assurance (Møller and Birkhead 1991). In Common Murres, high attendance of males in the colony during the pre-laying period has been suggested as a mechanism of both site and mate defense, as well as a means of obtaining EPCs (Birkhead et al. 1985; Wanless and Harris 1986; Hatchwell 1988).

In this study, cuckolded males did not spend less time in the colony than non-cuckolded males. This suggests that high attendance of the male at the site was not sufficient to deter his mate from accepting EPCs if she chose to do so, a result similar to that found for Razorbills (Wagner 1992*d*), a closely-related species of the same family, Alcidae (Bédard 1985; Moum et al. 1994), which may rarely hybridize with Common Murres (Wilhelm et al. 2001). In absolute terms, females accepted more copulations when their mate was absent (14/17 successful EPCs) than when he was present (3/17 EPC successes), although they had more opportunity to do so when he was absent (i.e., there were 49 EPC attempts on females in their mate's absence versus 16 when their mates were present). Also, marked females that solicited EPCs most often did so in their mate's

absence. The notion that male site attendance prevents females from EPCs is also not supported by the observation that two females completed successful EPCs with their mates present (Section 2.5.1.). Indeed, Birkhead et al. (1985) reported one such case of a female accepting an unforced EPC while her mate was present, although in Hatchwell's (1988) colony, successful EPCs only occurred while the females' mates were absent.

One interesting difference between Common Murre and Razorbill mate guarding behaviour is that, in the present study, male murres never attempted EPCs when their mate was present on the ledge. This contrasts to male Razorbills that performed EPC attempts while their mate was present in the mating area (and which were often prevented from completing an EPC by their mate's interference; Wagner 1992*b*). Birkhead et al. (1985) and Hatchwell (1988) did not report female mate presence during male EPC attempts, either because it was unknown, or not deemed to be a significant factor. An argument could be made in this study that female attendance was a more effective form of mate-guarding males than male attendance was for mate-guarding females. However, there was no evidence that females attempted to directly prevent their mates from EPCs in any manner. It is more likely that when the female was present on the ledge, pair behaviours, i.e., preening, displaying and copulating, took precedence over the EPCs attempted by males. The significant positive correlation between the ratio of females to males and PC activity supports this; when there were high numbers of females on the ledge, individuals were involved in pair, not extra-pair, interactions.

Overall, for males at least, attendance in the colony did not appear to be an effective mate-guarding strategy. Acceptance of EPCs was solely under the control of females and males with lower overall attendance were not cuckolded more than males with high attendance. Females whose mates were present were subjected to fewer EPC attempts, suggesting that males avoided EPC attempts on a female whose mate was nearby, possibly to avoid subsequent aggressive interactions with the male. Males that spent more time in the colony tended to make more EPC attempts (significant in 2000; $p<0.056$ in 1998 and 1999). This could indicate that the function of high male attendance is three-fold: it permits site defense (Wilhelm et al. 2000), it ensures that the male is present when the female returns to the colony in order to perform pair copulations (and increase his probability of successful paternity), and it allows the male to at least have the possibility of attempting EPCs while his mate is away. The hypothesis that high male attendance is a form of mate-guarding in the Common Murre was not supported in this study.

2.5.4 Female Control of Copulation Outcome

Not surprisingly, if copulations were initiated by females, they almost always resulted in cloacal contact. This was true for both PCs and EPCs. Hatchwell (1988) found a similar result: in the type of copulation which he called unforced EPCs, defined by female solicitation and/or cooperation, there was a 95% success rate. This was higher than success rates for both PCs and forced EPCs (74% and 6%, respectively). Birkhead's

(1985) & Hatchwell's (1988) forced EPCs likely equates to EPC attempts in the current study, which, here, were always unsuccessful by definition. I cannot reconcile why 6% and 5% of resisted EPCs resulted in successful cloacal contact in Hatchwell's and Birkhead's studies, respectively, while none did so in the current study. Birkhead et al. (1985) stated that determining the success of FEPCs in the field is very difficult. However, perhaps because our blind was located much closer to the observed birds (< 3m from all pairs) than either Hatchwell's blind (60m) or Birkhead's blind (15m), we were able to determine cloacal contact with more certainty and/or accuracy.

While I intended to use the terminology of Birkhead (1985) (borrowed from McKinney's et al. 1984 study on waterfowl), a period of time observing pair and extra-pair copulations indicated that successful cloacal contact was only likely to be achieved in this species if the female cooperated. No observer in this study has ever reported seeing a successful copulation that was resisted by the female, as the female can stand up and prevent forced contact. Even if she cannot stand immediately, she does not have to maintain the proper crouch position that seems necessary for both the male to balance on her back and for her to raise up her lower body slightly to facilitate cloacal contact. Similar requirements for female cooperation are seen in all species that lack intromittent organs (Briskie and Montgomerie 1997; *cf.* Castro et al. 1996 who describe unusual forced face-to-face copulation in the Hihi (or Stitchbird), *Notiomystis cincta*, which females seem unable to resist). As male murres do not have an intromittent organ (but waterfowl do; McKinney et al. 1984), it is extremely unlikely (if not impossible) that forced EPCs will ever result in insemination in this species (Fitch & Stugart 1984;

Wagner 1991*b*; *cf.* Birkhead and Møller 1992). Indeed, this is also the reason why multi-male EPC attempts were considered separately from single-male EPCs. Such attempts were always resisted and seemed extremely unlikely to ever be successful. The fact that the duration of these multi-male attempts was similar to that for unsuccessful PC and EPC attempts supported this view; indeed, it must be admitted that it is highly improbable that a male could balance on the female's back and make cloacal contact in approximately 5 seconds, especially with one or more males on top of him.

Recognition that females may exercise pre-copulatory, post-copulatory, and *in copula* control of fertilization, by mate choice, sperm selection, and copulation behaviour, respectively, has grown over the past decade or so (e.g., Birkhead and Møller 1993; Birkhead 1998*b*; Jennions & Petrie 2000). Most workers would likely concur that, at a minimum, behavioural acceptance of a copulation is under complete female control in most species of birds which have "lost" an intromittent organ (Briskie and Montgomerie 2001). Thus, by deciding who to copulate with and when to copulate with a particular male (e.g. employing a behavioural decision rule such as "copulate last with the male you want as a sire"; Briskie and Montegomerie 2001), a female can determine the paternity of her chick. Other mechanisms of post-copulatory, pre-fertilization female control of paternity include mechanical ejection of sperm from the cloaca, and the more controversial cryptic female choice or sperm selection in the female's reproductive tract (Birkhead and Møller 1993; Keller and Reeves, 1995; Birkhead 1998*b*; Jennions and Petrie 2000). It should be noted that while ejection of sperm may or may not be under the female's *conscious* control, cryptic sperm selection implicates physiological mechanisms

that have evolved under sexual selection and are presumably not available for conscious control by the female.

The fact that, in at least two years, female attendance at the ledge was generally higher for those individuals which accepted EPCs than for those that did not further supports the belief that females controlled copulation activity. Assuming that females were not differentially constrained in terms of the amount of time that they could spend in the colony during pre-laying, it appeared that they could modulate their exposure to EP activity by either coming to or staying away from the ledge. Females which sought out EPCs, either by direct solicitation or by making themselves available for EPC attempts by males and subsequently accepting them, needed to be present more often in the ledge when the female mates of their extra-pair suitors, and/or their own mates (if they were paired), were absent.

Interestingly, all cases of recognized extra-pair paternity occurred in 1998, indicating that EPCs definitely occurred during the fertile period of at least three females, two of which were not observed taking part in successful EPCs (there were no behavioural observations on the third unmarked female (93F) in the pre-laying period). This could indicate that 1) the fertile period of these females began before observations commenced that year, at 19 days prior to the first egg being laid in the ledge, 2) the EPFs occurred in the absence of observers after May 27th, when pre-laying observations ceased, or 3) EPCs occurred on days when there were no observations recorded. The last

possibility is remote, as observers went to the site every consecutive day from May 3-May 27, leaving only after several hours if no murres came to the ledge.

In support of the first possibility, Hatchwell (1988) suggested that the female's fertile period could extend to 25 days prior to egg laying. The estimated lay dates of the three females with EPP chicks were June 19 (84F), June 5 (93F), and prior to June 3 (6F; an exact date could not be determined, as the chick hatched prior to the re-commencement of observations on July 5th). Given the late date of the egg-laying for 84F, it is likely that the chick produced was the result of a second egg laid. Thus, for these females, there were between six to 23 days after observations ceased and before their eggs were laid during which each female would have been fertile and successful EPCs (and EPFs) could have occurred. This possibility seems a plausible explanation for these cases of EPP in the absence of EPC behaviour, although given that EPC successes tended to occur earlier (i.e., the "Before Peak" period) in 1998, it is also plausible that 6F and 93F participated in successful EPCs prior to the beginning of observations. Other studies in which EPP rates were detected in the absence of EPC behaviour have offered alternative explanations such as the possibility that the EPCs occured more furtively or at other (unobserved) locations (Birkhead & Møller 1992; Lifjeld et al. 1993). Such possibilities seem remote for Common Murres but cannot be completely excluded, as Common Murres have been rarely spotted visiting other colonies during the pre-laying period (Halley and Harris 1993).

It is worth noting, again, that in the year of higher PC activity (1998), two females that had chicks of extra-pair paternity refused all PC attempts by their mates. This might suggest that these females' refusal of PCs were a behavioural means of ensuring that extra-pair male(s) fertilized their eggs. It is interesting that, given the likelihood that a "last male" advantage in sperm competition exists for most birds (Birkhead and Møller 1998), little attention has been paid to the possibility that a female must modify her PC acceptance rate, or, certainly, the timing of her acceptance of PCs relative to EPCs, if EPCs are to produce EPFs (i.e., if they are performed for genetic benefits). Thus, if a behavioural propensity to seek "good genes" by way of performing EPCs has evolved, it seems necessary that a modification of PC behaviour would have co-evolved, at least in those species in which a female cannot be sure if she will have the opportunity to mate again with the EP male (or any other male), following the acceptance of copulations from her mate, i.e., species in which EPC rates are relatively low. It does seem likely that the complete refusal of PCs is unusual, as this could possibly have large costs for the female, either in terms of abandonment or reduced paternal investment (e.g., the reverse of the potential benefits of multiple PCs: Hunter et al. 1993). Conversely, for species in which EPC rates are high, and, thus, access to EP mates is relatively unrestricted, refusal of PCs may not be necessary for an EPF to occur. As long as the female does not copulate with her mate following her last possible EPC prior to ovulation, she will probably obtain an EPF. Females of species with small clutches, especially with a clutch of one, may also be more likely to show modifications of PC rates or timing of PC acceptances, if they perform EPCs for genetic benefits. In species that perform EPCs and produce multiple-egg clutches, paternity is generally mixed (Birkhead and Møller 1995); i.e., the pair male

and extra-pair male(s) will have sired different chicks in the same brood. In this scenario, females may not be under so much pressure to alter their PC behaviour, as long as they can assure EPP for some of their chicks. However, in species such as the Common Murre, that produce one chick per year, and have relatively low EPC rates, females may have to alter their pair copulation behaviour if EPCs are performed for genetic benefits. Modification of the PC rate to ensure EPFs would also be expected if the "proportional representation" hypothesis of sperm competition operates in a species (Birkhead et al. 1985). In this case, the timing of the female's acceptance of PCs relative to the EPC should not be critical, but the total proportion of PC acceptances relative to the number of successful EPCs (with the preferred male) would be.

2.5.5 Pair Bond Stability and EPCs

The most compelling relationship of any factor examined in this study to EPC successes by females was pair bond stability. Overall, females that divorced at some point from 1997-2001 were relatively more likely to have performed successful EPCs than females that were in stable pair bonds. Somewhat surprisingly, male EPC activity was unrelated to the stability of the pair bond. Thus, there was a decoupling by sex of the relationship between performance of successful EPCs and pair bond stability, suggesting that EPCs could have different functions and/or consequences for males and females. A more detailed examination of the unstable or divorced males and females and the patterns of their EPC behaviour could illuminate whether this is so.

Moody (2001: Moody et al. 2001) examined the seven divorces (in which pairs separated and both partners were seen after and known to be alive) occurring over the course of this study and found that divorced birds had significantly lower reproductive success before divorce compared to both widowed birds (in which mates were not seen again) and reunited pairs. She further classified the divorced individuals as either choosers or victims (as per Ens et al. 1993). Choosers, those individuals that initiated the divorce typically by leaving the site for another site occupied by a recently widowed bird with previous reproductive success, had increased reproductive success following the divorce, while victims did not.

I examined the EPC behaviour in individuals from six divorces, in order to determine when EPCs occurred in relation to the timing of the divorce, and how the individual's status as choosers or victims was related to the performance of EPCs. The divorce of 22F was excluded from this analysis, as she had new mates in each consecutive year (Section 2.4.20(7)). Complete behavioural observations (i.e., before and after the divorce) existed for three female choosers and three male choosers, but for only two male and two female victims, as one female (89F) and one male (21M) left the ledge during or following their divorces.

Victims: Male victims (3M, 93M) had successful EPCs *following* the divorce only; neither EPC attempts nor EPC successes were recorded for these males in the years prior to their divorce. Female victims (84F, 12F), however, had successful EPCs both *before* and *after* their divorce. Although an EPC was not observed, 84F produced a chick of extra-pair paternity in 1998 (and was divorced the following year). Recall that 12F had a

successful EPC in her mate's presence two years prior to her divorce. Both females had EPCs after their divorce, although they were technically without mates when doing so (i.e., they copulated with paired males).

Choosers: Male choosers (12M, 84M, 89M) had EPCs *only* in the year of (i.e., during or after) their divorces. 89M participated in EPCs with an unmarked female, as well as with his future mate (21 89F) in 1998, the year of his divorce. The other two males, 84M and 12M, were not observed to have performed EPCs until they began their move to their new mate's site, i.e., all EPCs were with their new, chosen mates. None of these males engaged in EPCs with any female following the formation of their new pair bond. In contrast, female choosers (3F, 21F, and 93F) all performed EPCs well before their divorce, with only 21F performing EPCs after she had formed a new pair bond. Thus, female choosers, but not male choosers, might have used successful EPCs as a means of mate sampling (Heg et al. 1993). It is certain that for 21F, successful EPCs occurred with her future mate, as well as with other males. 93F, who had an EPF in 1998 (and, thus, had at least one EPC that year), was seen performing an EPC in the year of her divorce with an unmarked male, who was likely her future mate. 3F was never observed to have performed EPCs with her future mate prior to her divorce.

One of the many hypotheses of divorce in socially monogamous birds (all reviewed in Black, 1996) is the "better options hypothesis" (Ens et al. 1993), which states that divorce often occurs when an individual leaves a mate of lower quality for one of higher quality. There is some support in this study for this hypothesis of divorce in Common Murres. Specifically, prior to divorce, choosers' rates of fish delivery to chicks

(adjusted for sex and year), an index of parental quality, were average, while the fish delivery rates of victims were below average (Moody et al. 2001). Thus, it appeared that choosers attempted to "trade up" to a higher quality mate. Indeed, this strategy proved successful, as the reproductive success of choosers did increase following divorce, and 5/6 of them no longer performed EPCs after pairing with their new mates was established. The one chooser which continued to perform EPCs after re-pairing was 21F, whose divorce was due to her mate being kept off the ledge by the resident Razorbill (Walsh et al. submitted). Thus, this female, who left her site and paired with 89M, might not have divorced "by choice". Rather, her pairing with 89M may have been an attempt at making the best of a bad situation and 89M could be a sub-optimal mate for this female. Most victims of divorce, on the other hand, did perform EPCs following their divorces.

It is interesting that a male's quality, as measured by chick feeding rates, was unrelated to the performance of EPCs by his female mate. Thus, low male quality *per se* was not a major factor in the proclivity of females to either perform EPCs or become divorced: rather, it is likely that the relative quality of both mates (Petrie and Hunter 1993) interacted to affect their pair bond situations and EPC behaviour by males and females.

While the relationship between female EPC behaviour and female quality is debated in species for which there is variation in the need for male parental assistance to successfully rear young (e.g., Gowaty's 1996*a* "constrained female hypothesis"; also Gowaty 1996*b*), even high quality female murres require male assistance, as a single

murre has never successfully raised a chick alone, and it is the male parent that attends the chick for several weeks after it has left the colony (Gaston and Jones 1998). Thus, if a potential cost of a detected EPC is lowered male investment, both high and low quality females should avoid EPCs. If a cost of detection of EPCs is increased risk of desertion by her mate, it would be expected, perhaps, that mostly low quality females should avoid EPCs, as their chance of obtaining a new mate would be less than that for a high quality female.

There is no evidence in this study that low quality females (i.e., female victims of divorce) avoided EPCs prior to divorce. While it could be argued that subsequent divorces of the two female victims were in retaliation for their EPC behaviour, this seems a somewhat unlikely, albeit intriguing, possibility. Even though the EPC of one female (12F) was conducted in front of her mate, she was not divorced until a vacancy appeared at a neighbour's site two seasons later. Interestingly, the second female victim (84F) had an EPP chick in the year prior to her divorce. Arguably, then, her EPC could have been detected if her mate somehow recognized that he was not the sire of this chick. Such recognition could operate through a mechanism such as phenotype matching (i.e., "the armpit effect", Dawkins 1982; possible support in Petrie 1999; *cf.* Griffith 1999 and Leonard et al. 1995) or through the male's "memory" of 84F's refusal of his pair copulations attempts in the year that the chick was produced, i.e., if the male was able to detect and recall that cloacal contact was not made with his mate, then he would "know" that this chick was not genetically related (an extension of one mechanism by which male Dunnocks, *Prunella modularis*, may assess paternal certainty, Davies et al. 1992).

Interestingly, the male was ranked as "below average" for his chick feeding rate in that year, although this could reflect his quality and not his paternal investment, as there was no other year in which this pair successfully hatched a chick to compare his rate with. It is worth noting that, due to their longevity, Common Murres are a species in which males would be expected to have a low tolerance of EPP and abandon their mates in response, assuming that they could accurately assess the parentage of their mates' offspring (Mauck et al. 1999).

An intolerance of EPFs might be further accentuated by the male's high parental investment in murres, i.e., his provisioning the chick at sea. In this study, two pairs divorced after they raised an EPP chick, although in the third case of EPP, the male remained with his mate (pair 6; see Chapter 4 for an alternate explanation of this continued pair bond). Anecdotally, for the pair that did not divorce (pair 6), the (EPP) chick's fledging was delayed; the chick was more than 23 days old, and quite large, when it finally left the colony with the male. In retrospect, it is tempting to consider the possibility that 6M had detected that this chick was not his offspring, even though he provisioned the chick well. Overall, though, these cases provide weak support for the notion that EPPs were detected and retaliated by the pair males, particularly since in one of the divorces (pair 93), the female was the individual who initiated it (i.e. 93F was designated as the chooser).

Male victims of divorce made no EPC attempts prior to their divorce, possibly because low quality males did not (or could not) perform EPCs and maintain a pair bond

simultaneously. Both of these male victims had attendance in the ledge prior to their divorce that was average (3M) or above average (93M) relative to other males, suggesting that they were not without the opportunity to attempt EPCs. It is possible that they lacked the experience or energy to perform EPCs in these years. However, following divorce, these male victims did re-mate, and were seen performing EPCs with females other than their new mates. Thus, one possibility is that their new mates were of lower quality relative to other females and EPCs were used to try to forge social bonds with higher quality females or to fertilize their eggs, whereas their old (pre-divorce) mates were of relative higher quality than other females, thereby preventing the males from EPC behaviour in some way. Alternatively, the intrinsic quality or experience of these males might have increased over time, making them both better able to attempt EPCs and more acceptable to females as an extra-pair mate (e.g., the relative attractiveness of an individual does not necessarily stay constant over time: Petrie and Hunter 1993).

It has been predicted and subsequently shown that females generally perform EPCs with high quality males (e.g., Blue Tits, *Parus caeruelus*, Kempenaers et al. 1992; Black-capped Chickadees, *Parus atricapillus*, Ramsay et al. 2000; see also Birkhead and Møller 1992, 1998), and that they may seek EPCs to obtain viability genes from males of high immunocompetence for their offspring (Møller 1997; Johnsen et al. 2000). In this study, males which were successful at obtaining EPCs in more than one year were generally older, experienced and successful breeders. Choosing a highly attractive or viable male for an extra-pair partner makes sense, particularly if the function of EPCs is to obtain indirect genetic benefits for offspring, as appears to be the case for the

aforementioned species. However, if the function of EP behaviour in a species is not exclusively to seek good genes (Ens et al. 1993; Heg et al. 1993), then females might not be so choosy about the quality of their EP mates. For example, if a female of low quality has a higher relative risk of mate desertion and EPCs serve to forge social bonds with possible future mates, the quality of her EP male may be unimportant (i.e., a low quality mate would be better than no mate if she was deserted). Exactly how and when a female assesses the quality of an EP mate, as well as the completeness of her knowledge is not clear, although there is evidence that honest signals of quality (e.g., morphological features and/or behavioural displays) are used (Slagsvold and Lijfeld 1997).

In inter-specific comparisons, Cezilly & Nager (1995) found that high rates of EPP were positively associated with high rates of divorce between breeding seasons in socially-monogamous birds, even when adult survival rate was controlled for as a confounding variable. They interpreted EPCs and divorce as different behavioural solutions to intra-sexual competition for limited breeding opportunities, i.e., EPCs and divorce are both means of "trading up" genetically and/or socially. However, they acknowledged that the mate sampling hypothesis (Heg et al. 1993, Colwell and Oring 1989), in which females evaluate breeding options through EPCs, provides an alternative mechanism for the association between EPP and divorce. Recent evidence in Black-capped Chickadees supported the former hypothesis: Chickadees used divorce and EPCs as separate strategies to obtain better mates and better genes (Ramsay et al. 2000). In Chickadees, there was no indication that females divorced in favour of extra-pair males, suggesting that EPCs did not function to facilitate mate change. However, upon careful

consideration, it does not seem reasonable to assume that the mate sampling function of EPCs would evolve for short-lived species such as passerines. Rather, the function of EPCs as facilitating mate change between years would be more likely to evolve in long-lived species that breed for many years, and in which there is a high probability of interacting with the same individuals/neighbours for many years, i.e., high site fidelity and philopatry. Thus, an association between EPP rates and divorce rates could exist for different reasons in different species, specifically, mate sampling in long-lived species and as a means of obtaining genetic benefits in shorter-lived ones. Furthermore, among-species differences in yearly clutch sizes (multiple versus single eggs) may influence the functions of EPC behaviour.

We do not yet fully understand the true nature of the relationship between EPC behaviour and divorce in Common Murres. Certainly, this relationship appears to be influenced by sex and individual quality or attractiveness (for which my only independent measure in this study is parental feeding rate which, admittedly, is likely insufficient to capture total variation in individual quality). In females that chose to divorce, there is some support that EPCs were used to sample more than one male prior to divorce, although there is no such evidence for males that were choosers of divorce. However, female choosers and victims may have participated in EPCs prior to divorce to either sample potential future mates, and/or form potential social bonds for the future should they be deserted, and/or in response to low reproductive success as a means of obtaining either sperm (in case of male infertility) or "good genes". Disentangling these reasons for a given female's performance of EPCs in the field is difficult, and likely impossible, as

any particular female may have more than one reason for accepting EPCs. However, for males, it seems fair to state that the EPCs of both victims and choosers were probably performed in the search for a new mate and/or as a strategy to obtain a possible EPF.

2.5.6 Why Do Female Murres Perform EPCs?

There are three likely reasons for the acceptance (and solicitation) of EPCs by female Common Murres that are not mutually exclusive: 1) facilitating mate change, 2) fertility insurance, and 3) obtaining indirect genetic benefits. It should be emphasized that the functions of EPCs are very difficult to sort out for any given female, and it is possible that EPCs occurring in any population simultaneously fulfill all functions for different females, or even, perhaps, for a single female. I contend that, in Common Murres, EPCs will fulfill "some of the these functions all of the time and all of these functions some of the time" for different individuals. I have attempted to evaluate these theoretical reasons for female EPC behaviour by referring to the individual circumstances in which females were observed to have accepted EPCs.

1) *Facilitating Mate Change*: The fact that females that divorced were more likely to have successful EPCs provides some support for the notion that one likely function of EPCs in the Common Murre is to facilitate mate change. Some of this EPC behaviour was seen prior to divorce, possibly to fulfill a mate sampling function as per Heg et al. (1993), while, in some females, it was also observed after divorce while the females were unpaired and, presumably, attempting to form a new pair bond. Similar patterns have

been reported for two other long-lived non-passerines: the Spotted Sandpiper (*Actitis maularia*, Colwell & Oring 1989) and the European Oystercatcher (*Haematopus ostralegus*, Heg et al. 1993).

In an early five-year study of Spotted Sandpipers, a species in which females are polyandrous and males provide most of the parental care, Colwell and Oring (1989) showed that females that participated in EPCs likely did so to assess and acquire future mates. Extra-pair mates of one year were likely to become pair mates in the subsequent year if the female switched males. A similar result was obtained for the socially monogamous Oystercatcher (Heg et al. 1993). In this species, both EPC and EPP rates were low, and the performance of successful EPCs predicted which individuals would become future partners. Birkhead (1998*a*) contended that these cases in which EPCs have direct benefits to females (and/or males) by facilitating mate changes across time are rather unique, and that, for most species, data support the pursuit of EPCs by females for the purpose of obtaining genetic benefits ("good genes"). This may be true, but one should be aware that the majority of EPC studies have been carried out on passerines (see Birkhead and Møller 1992, 1998). Indeed, the assertion that the relative absence of EPP in relation to the number of EPCs observed in many non-passerine species (e.g., Northern Fulmars, *Fulmarus glacialis*, Hunter et al. 1992; Purple Sandpipers, *Calidris maritma*, Pierce and Lifjeld 1998; Semipalmated Plovers, *Charadrius semipalmatus*, Zharikov and Nol, 2000; Lesser Kestrels, *Falco naumanni*, Negro et al. 1996, Villaroel et al. 1998; Humboldt Penguins, *Spheniscus humboldti*, Schwartz et al. 1999) points to the likelihood that EPC behaviour probably has different functions in such species. These differing

functions likely depend on the species unique life-history strategies. I submit that in long-lived species, particularly philopatric ones, female acceptance of EPCs likely functions, in part, to facilitate future mate change.

It is significant that there were many females (9/16) that performed at least one successful EPC, did not divorce, did not produce EP chicks, and did not suffer reduced reproductive success. Why should these females accept EPCs? In four of these nine cases (5F, 7F, 17F, and 24F), the only recorded EPCs occurred prior to the male's arrival in the colony (in fact, 17M never did return; Section 2.4.21). This is consistent with the "arrival asynchrony" hypothesis of divorce in Black-legged Kittiwakes (*Rissa tridactyla*) or the "musical chairs" hypothesis of divorce in Blue Tits (reviewed in Black, 1996), both of which state that pairs are at higher risk of divorce if they arrive in the breeding area at different times. More recent support for these hypotheses has been found in *Aptenodytes* penguins (Bried et al. 1999; Olsson et al. 2001). By extension of these hypotheses, it might be supposed that a female Common Murre arriving first at her site and finding her male absent would be more likely to accept EPCs, as she could not be certain that her mate had survived the winter. Thus, such EPCs for these females could facilitate forming a social bond with another male, in the event that her mate did not return. Indeed, a vacancy left by the non-return of an individual was a common trigger for a neighbour's divorce. If these four females were of high quality relative to their extra-pair mates' females, then accepting EPCs from these males could be the equivalent of "luring him over" as a prophylactic measure should their mates not return. Since these four females did not show any further EPCs once their mates arrived at the ledge, it would be predicted

that their males were of high quality. In three of the four cases (5M, 7M, and the new 17M), the fish delivery rate of these males to chicks aged 1-12 days was above average, likely indicating high parental quality, while the final male was rated "average". Thus, following the arrival of their mates, EPCs were no longer strategic or necessary for these females.

The remaining five females from stable pair bonds that accepted EPCs also had high reproductive success, but performed successful EPCs *after* having reunited with their mates. These females also accepted PCs following their EPCs. Since none of these pairs have divorced, it seems less likely that the purpose of these EPCs was to facilitate mate change, unless the females were uncertain of their mates' likelihood of remaining in the pair bond. Rather, fertility insurance and/or seeking genetic benefits are more likely functions (discussed below).

There are two cases in which females have had at least one successful chick with their social mates (determined to have been sired by their mates), then subsequently had EPCs and experienced hatching failure. One female (12F) was deserted by her male, while the other (6F) remained in a stable pair bond despite an EPP chick and subsequent egg failure, and continued performance of EPCs.

As described previously, 12F accepted an EPC in her mate's presence in 1999. This EPC was followed by PCs, but their egg did not hatch that year. It is possible that the egg was infertile, but this seems unlikely, as the pair successfully hatched an egg in the

previous year. It is likely that 12F performed this EPC to facilitate mate changing, as no PCs occurred prior to the EPC, and it may have appeared to her from the lack of interest of her mate that she was at risk of desertion, or was, in fact, already deserted.

Heg et al. (1993) found that the length of time that Oystercatcher pairs had been together was negatively related to their probability of divorcing, and, hence, their performance of EPCs. The fact that younger birds are more likely than older birds to be in pair bonds of relatively shorter duration predicts that EPCs might be disproportionately observed in the younger individuals of a population. However, as it has been shown that females often seek out EPCs with older males, particularly when age is correlated with resources, but sometimes even when it is not (e.g., reviewed in Table 1, Brook and Kemp 2001), one might predict that the older males in a population, in particular, should show a higher proportion of successful EPCs. For the Common Murres in this study, I do not have complete data on the length of pair bonds, but can state with certainty for some pairs and individuals that they are older (i.e., the pairs existed or the individuals were breeders since at least 1996 or 1997) while others are newer (i.e., existed or bred since 1998). It is interesting that all of the males from which females accepted EPCs in more than one year were in the older category (Section 2.4.22). However, there was no apparent relationship between relative age and female EPC behaviour.

2) *Fertility Insurance*: It seems plausible that, from a female's perspective, the purpose of EPCs that occur after a stable pair has reunited for the season is fertility insurance against the possible infertility of their mates. In this study, five stable females performed such

"after-mate-reunion" EPCs, although the number of accepted EPCs was low. Three females (11F, 9F, "old s9"F) each had a single successful EPC in only one year. Two others (23F, 25F) each had several EPCs after a successful reunion with their mates, with one female (25F) having these in two different years. If these females accepted EPCs to facilitate mate changing, it might be expected that their male mates would be of lower attractiveness or quality than average. There was no indication from available fish delivery rates to chicks data, an index of parental quality, that these males were below average relative to other males. If these females were accepting EPCs to obtain "good genes" from an EPF, it might be expected that each female would lower her PC acceptance rate (behavioural control of paternity) or produce a chick of extra-pair paternity (sperm selection or sperm competition). All but one of these females were seen to have accepted PCs in the days following their EPCs (the exception being 23F who could not be observed following her EPC). Assuming that additional EPCs did not occur prior to egg-laying, it would then be expected that these females' chicks were sired by their social mates. For 11F, this is indeed the case, as the chick she produced in 1999 was likely to have been sired by 11M (see Chapter 3), and her successful EPC had been followed by successful PCs. Paternity analyses could not be carried out on chicks from the other four females in the year of their EPCs.

An alternate explanation for the EPC behaviour of these five females is that they opportunistically accepted EPCs for the *potential* of indirect genetic benefits in the absence of complete information about the quality of the EP males (see Slagsvold & Lifjeld 1997 for discussion of "Incomplete Knowledge Hypothesis" as an explanation of

variation in EPP rates). If the females accepted the EPCs from males who potentially could have better genes than their own mates, the females could reserve the option of preventing the sperm that may have been stored during the EPC from fertilizing their egg by accepting many PCs from their mates. One interesting possibility is that the performance of the copulation itself may be a means of assessment by the female (and/or male), a mechanism that has been suggested for Razorbills (Wagner 1991*a*). It is possible that the quality of the male's behaviour or less observable cues that may be detected by the female, such as volume of ejaculate, would contribute to her assessment of his suitability as the sire of her chick. However, if EPC behaviour is costly to the female (as has been assumed, Hunter et al. 1993; Gowaty 1996*b*), the use of copulation *per se* for mate (or genetic quality) assessment might not be expected to evolve readily, unless the pay-offs of obtaining the best possible genes (from either your mate or an EP male) are much higher than the potential risks of EPCs.

Is it possible that the four females that were in stable pair bonds and only accepted EPCs before they were reunited with their mates did so for reasons of fertility insurance versus "non-return of mate" insurance? If the EPCs which these females accepted were with relatively attractive/ high quality males, storing sperm might serve the purpose of ensuring fertility, particularly if the female's mate did not return, and she was forced to pair with a sub-optimal (potentially infertile) male during that season. However, the role of these EPCs as facilitating mate change, should it have become necessary, seems more likely.

One case study (6F), provides an interesting example of the difficulty in separating the functions of EPCs for any particular female. Pair 6 has not (yet) divorced, is known to be old (both 6F and her mate were banded in 1986 as breeders), has had one known case of EPP during the year in which 6F refused PC attempts from 6M, and, subsequent to that, has failed to produce eggs that hatched. Only in the first two years of this study, 1996 and 1997, did this pair successfully raise a chick that was determined to be 6M's genetic offspring. Furthermore, 6F has had EPCs followed by PCs in two of the years that eggs have failed to hatch (1999 and 2001). In 2000, this pair's egg also did not hatch, and only successful PCs were observed in this year. These hatching failures occurred in spite of continuous incubation by the pair. Assuming that, in murres, the last male to inseminate the female will fertilize her egg, this pattern of non-hatching eggs suggests that the male's sperm was either not viable (but had displaced the previous EP male's sperm), and/or that the pair produced chick embryos with lethal alleles causing pre-hatching mortality (Koenig 1982). Unfortunately, we were unable to take the eggs of this pair to determine whether fertilization had, in fact, occurred (as per Birkhead et al. 1995).

It is possible that 6F performed EPCs to guarantee fertility, although no EPCs were seen in one year (i.e., the Peak period of 2000). If obtaining good genes was the function of all her EPCs, the subsequent performance of PCs following the EPCs seems to have negated their purpose. If the female's refusal of PCs with her mate in 1998 following an EPC (which led to an EPF) was to ensure that the extra-pair male fertilized her egg, it is unusual that she accepted PCs from her mate after she had performed EPCs

in two other years (i.e., if she wanted "good genes" in one year, why not in all years?). One possible explanation for this is that the males with whom she copulated in the years during which she accepted PCs following her EPCs could have been less attractive or of lower quality than 6M, but that the EP male in 1998 was of higher quality. If this is so, the purpose of accepting an EPC, and subsequently refusing all PCs in 1998, was to obtain "good genes" while, in the other two years, the purpose of the EPCs could have been merely to ensure fertility. The data circumstantially support this notion: of the two males whose EPCs were followed by PCs, one was unmarked (i.e., not a resident of the ledge) and the other was ranked lower for parental quality than 6M. Also, one potential father of the EPP chick, 5M (see Chapter 3), was ranked consistently as "above average" for parental quality.

Alternatively, as discussed above, if the female's assessment of her extra-pair mates' quality did not occur until the EPC was completed, the original purpose of all 6F's EPCs might have been to ensure fertility. If her subsequent assessment of the EP males' quality relative to her own mate was that they were inferior in two years, she might have decided to accept PCs from her mate in these years. Thus, this female's copulation pattern may have been affected by the quality of available EP mates in different years and by the completeness of her knowledge of their quality (Slagsvold and Lifjeld 1997).

It is interesting that, in the case of pair 6, the pair's non-hatching of eggs might be due to pre-hatching chick mortality from genetic defects, as the coefficient of relatedness for these individuals is high (>0.5 or full-siblings: Chapter 4). In other bird species,

decreased hatchability of eggs has been related to close inbreeding of relatives (Bensch et al. 1994; Kempenaers et al. 1996). Furthermore, in Tree Swallows, *Tachycineta bicolor*, there is evidence that females who did not have extra-pair chicks in their nests had more hatching failures due to embryo mortality than females with extra-pair young, suggesting that females sought EPCs due to genetic incompatibility with their mates (Kempenaers et al. 1999). However, there is no reason to assume that a female can be "aware" that a male's sperm viability or genetic compatibility with her is low and compensate behaviourally for this in Common Murres.

3) *Indirect genetic benefits*: It has been argued that "multiple mating for purely non-genetic benefits is unlikely as it invariably leads to the possibility of genetic benefits as well" (Jennions and Petrie 2000). Indeed, there is no reason to assert that the function of EPC behaviour in any species is solely for non-genetic or direct benefits. As a corollary, there is also no reason to assume that the indirect genetic benefits of EPCs are the singular force behind the evolution of EPC behaviour in general (as seems to be proposed by Birkhead and Møller 1998). Mounting empirical evidence for non-passerines and even some passerine species (e.g., the Great Tit: Lubjuhn et al. 1999) suggest that factors other than the genetic quality of males have important roles in determining the extent of and variation in EPP.

In this study of Common Murres, there is some evidence that females accepted EPCs for the purpose of obtaining fertilizations in at least two cases. These were the cases in which females refused all PC attempts from their mates and subsequently produced a

chick of extra-pair paternity (6F, 84F). In the third case of EPP (93/95F), the PC behaviour of the pair in the pre-laying period was unknown; however, this female subsequently divorced her social mate in the year following the EPF, so it possible that, in this case, EPC behaviour may have also served the function of facilitating mate change in the future. As has been argued (Section 2.5.5), it seems likely that if a female accepts EPCs for genetic benefits, she would simultaneously alter her PC acceptance pattern. For 6F and 84F, this pattern was seen, indicating that the function of EPCs for these females in the year that EPP occurred was most probably to obtain an EPF.

As discussed previously, other females which accepted EPCs but then later accepted PCs might have done so *initially* to get potential indirect genetic benefits from the EP males. However, these female's decision to accept PCs and effectively negate the possibility of an EPF could have been mediated by their subsequent assessment of the EP mate's quality relative to that of their own later-returning mates.

2.6 Conclusions

To a large extent, females control both pair and extra-pair copulation behaviour in Common Murres. EPC behaviour of females is multi-faceted; it likely has more than one function in any given species, and may have more than one function for any given female at a particular time. Factors such as the female's quality, the quality of her social mate in relation to other available EP or social partners, the circumstances of her pair bond (e.g. whether she is mismatched with her mate in terms of quality and at greater risk of

desertion and how long the pair bond has existed), and her cumulative reproductive success probably have major influences on an individual female's propensity to engage in EPCs. Exactly how such factors interact with each other and whether they result in EPC behaviour, or are just correlates of EP behaviour due to underlying factors is unknown and difficult to determine in the field.

In Common Murres, females show evidence of performing EPCs for the multiple purposes of facilitating mate change, ensuring fertility, and obtaining genetic benefits. Males, on the other hand, seem to perform EPCs in two contexts: 1) in order to increase personal reproductive success by obtaining an EPF (as would always be predicted) and 2) in order to establish a pair bond with a female if the male is unpaired or in the process of divorcing his mate. While pair bond stability was related to the female performance of EPCs, it was unrelated to male EPC performance. Males which divorced did not show high levels of EP behaviour and were observed to have copulations only in the context of forming a new pair bond. For some males that remained unpaired following divorce (i.e., the victims of divorce), this meant attempting to copulate with several different females. However, males from divorced pairs that were successful at obtaining a new mate (i.e., the choosers) generally showed only copulations with that female. Furthermore, only a few males were seen to have had successful EPCs in two or more years, and, in fact, relatively few even attempted EPCs in more than one year. Thus, while EPC behaviour for both male and female Common Murres seems to serve a purpose in terms of facilitating mate change or pair bond formation, the timing of EPCs by females relative to their divorce was different. Instead of occurring in the immediate context of a divorce,

females performed EPCs in years other than the year of the divorce.

How much of an assessment of a male does a female need to make prior to accepting an EPC from him? This question may be unanswerable. As most EPC attempts were refused by females, it might be assumed that some females were simply not interested in EPCs due to their favourable situations (i.e., high reproductive success with partner, a high quality mate, etc.). Alternatively, their assessment of the potential EP mates might have been low relative to their own mate's assessment and, thus, EPC attempts were refused. Other females accepted some EPCs from some males. In some respects, the EP behaviour of murres on the ledge was opportunistic; males attempted EPCs only when their mates were absent and females were more likely to experience an EPC attempt in their mate's absence. Given the unpredictable nature of when a female might receive an EPC attempt from a male, one strategy of a paired female who is inclined to accept some EPCs might be to "accept now, decide later", in terms of what to do with the EP male's sperm. If she "determines" that the EP male is of high quality or attractiveness, and, thus, his genes might confer a genetic advantage upon her chick, she might decide to alter her PC behaviour and ensure an EPF. If she determines otherwise, she is free to ensure that her mate sires her chick. If the female's acceptance of the EPC is not solely to obtain genetic benefits, but is to sample potential mates, the female might also be expected to opportunistically accept EPCs from some males. Indeed, a female may also solicit an EP male which she has assessed or is attempting to assess further.

In Common Murres, there were a relatively low number of successful EPCs, performed by a minority of individuals each year, which resulted in few EPFs (Chapter 3). Pair copulation behaviour was much more frequent and successful than EPC behaviour, but levels of PC behaviour fluctuated significantly over years, while overall EPC rates remained relatively stable. Changes in PC behaviour appear to be related to the attendance patterns of females in the colony, which, in turn, are likely influenced by (unknown) ecological factors.

It is unlikely that Common Murres perform EPCs for the sole purpose of obtaining indirect genetic benefits, a pattern that has been reported increasingly more often for other non-passerine bird species. While some have argued that most empirical support favours the evolution of female EPC behaviour due to the indirect genetic advantages that such behaviour confers on a female's offspring, it should be acknowledged that, at least for long-lived non-passerines, data also support the notion that female EPC behaviour has evolved for other non-genetic or direct reasons, i.e., facilitating mate changes and/or ensuring fertilization.

This study is added to the growing consensus in avian literature that, by and large, females control male's access to both copulation and paternity. It has also contributed information on individual variability in the performance of EPCs by Common Murres. Further research on the behavioural and physiological mechanisms by which this is accomplished is required to completely elucidate the nature of the relationships between female copulation behaviour, male behaviour, and life-time reproductive success.

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Chapter 3
Extra-pair Paternity in Common Murres: Evidence for Yearly Variation?

3.1 Abstract

To investigate the rate of extra-pair paternity (EPP) in Common Murres (*Uria aalge*), parentage analyses using four microsatellite loci (Ibarguchi et al. 2000) were conducted on 30 (out of 35) families of individually-marked murres from Great Island, Newfoundland, Canada over four breeding seasons (1996-1999). Results demonstrated three likely cases of extra-pair paternity (EPP) over the four years in this socially-monogamous, long-lived seabird, indicating an overall EPP rate of approximately 10%, with a maximum EPP rate, based on integrating behavioural observations with genetic analyses, estimated at 20%. Interestingly, all cases of confirmed EPP occurred in one year, 1998, a year characterized by comparatively high copulation rates in this population. In this study, three other cases of putative parent- chick mismatches occurred and are ascribed to adoption or alloparenting, chick misidentification, and the presence of a null allele mutation at one locus. Behavioural observations suggest that cases of EPP may be related to mate changing and insuring fertility in this population of Common Murres, as well as females seeking indirect genetic benefits. This study suggests that temporal variation in EPP rates may be particularly significant in long-lived species such as seabirds. I discuss the importance of conducting multi-year studies of EPP that incorporate extensive behavioural recording in seabirds.

3.2 Introduction

Paternity analyses in birds, made possible by the development of molecular techniques, have revealed that the proportion of extra-pair copulations (EPCs) resulting in extra-pair paternity (EPP) can vary widely among species (Birkhead and Møller 1995). The percentage of offspring sired by extra-pair males, the EPP rate, ranges from 0-76% in the more than 100 species that have been examined to date (Birkhead 1998). Among-species differences in EPP are presumably due to variation in pair bond stability, opportunities for females to gain direct benefits from extra-pair males (e.g., protection, food), the number of available mates, and the level of breeding synchrony in a population (reviewed in Petrie and Kempenaers 1998). Within a species, EPP rates can differ among populations and may be dependent on breeding density (Westneat and Sherman 1997) and population genetic variation (Petrie and Kempenaers 1998). However, these factors have not been able to account for all within-species EPP differences (e.g., the House Sparrow, *Passer domesticus*, Griffith et al. 1999).

Seabirds are generally long-lived species and individuals typically form lasting social pair bonds. They exhibit relatively low levels of EPP, even in species where EPCs have been observed (e.g., Northern Fulmars, *Fulmaris glacialis*, Hunter et al. 1992). There is no clear relationship between taxon and EPP rate in seabirds. Studies of Charadriiforme seabirds have demonstrated variable rates of EPP ranging from 0% in Caspian Terns, Herring Gulls (*Sterna caspia* and *Larus argentatus*, respectively; J. Quinn

cited in Birkhead and Møller 1992), and Western Gulls (*Larus occidentalis*: Gilbert et al. 1998), to 8.3% in Common Gulls (*Larus canus:* Bukacinska et al. 1998). The European Shag (*Phalacrocorax aristolelis*), a Pelecaniforme, had an EPP rate of 9.3% (Graves et al. 1992). Procellariiforme seabirds have also shown variable interspecific EPP rates. Studies of the Northern Fulmar (Hunter et al. 1992), Cory's Shearwater (*Calonectris diomedea:* Swatchek et al. 1994), and Leach's Storm-Petrels (*Oceanodroma leucorhoa*: Mauck et al. 1995) showed no cases of EPP in the populations examined. However, EPP was detected in another Procellariforme, the Short-tailed Shearwater (9-13%: *Puffinus tenuirostris*: Austin and Parkin 1996). The highest reported rate of EPP in any seabird is for the Waved Albatross (*Phoebastria irrorata)* in which 25% of all offspring were sired by a male other than the social male parent (Huyvaert et al. 2000). In general, the rates of EPP in non-passerine species are lower than for passerines (Fleischer 1996; Westneat and Sherman 1997). This could indicate that the primary purpose of EPC behaviour in such species is *not* to obtain extra-pair fertilizations (EPFs), as has been suggested for most passerines (Birkhead 1998; *cf.* Lubjuhn 1999, 2001), although other factors, such as differing clutch size among species, may also affect EPP rates.

It is important to note that many of these studies reporting EPP, or a lack thereof, were conducted in a temporally-restricted framework, i.e., typically one or two field seasons (*cf.* Swatchek et al. 1994). The present study on Common Murres (*Uria aalge*), conducted over four years, supports the notion that caution is warranted when interpreting EPP data, particularly in species with reproductive longevity such as seabirds. In fact,

among-year differences in population EPP rates have been recorded in at least three shorter-lived passerine species (Red-winged Blackbirds, *Agelaius phoeniceus*, Weatherhead et al. 1994, see also pers. comm. cited in Petrie and Kempenaers 1998; Bluethroats, *Luscinia s. svecica*, Johnsen et al. 1998; and Great Tits, *Parus major*, Lubjuhn et al. 1999). Variation in the levels of EPP for successive Great Tit broods *within* a single breeding season has been recently reported in the context of a five-year study (Lubjuhn et al. 2001). One drawback of a more limited study period for species which show (or might show) significant among-year variation in EPP is that a misleading impression of the true EPP occurrence in the species may be generated. Thus, any taxonomically-linked differences in EPP rates among species could be masked. In addition, long-term studies which incorporate behavioural analyses have the advantage of being able to examine the patterns of EPP among individuals and pairs over time; information that is critical to understanding the functions of EPCs in any given species (Lubjuhn 1999).

Common Murres are long-lived, socially monogamous seabirds that breed in large, dense colonies on cliffs and offshore islands in the Northern Hemisphere (Nettleship and Evans 1985). Each successful breeding pair produces only one chick per year. Breeding begins at 5-6 years of age and can ensue until death (>20 years of age; Gaston and Jones 1998; CJW, SIW, AES, unpubl. data). Common Murres are philopatric and adults typically re-occupy the same site each breeding season (Gaston and Jones 1998). Adult mortality is low (~6% per year), pair bonds are long-lasting, and divorce

rates are low, reported at approximately 12% (M. Harris, pers. comm. in Black 1996). Nevertheless, Common Murres have been observed performing EPCs (Birkhead et al. 1985; Hatchwell 1988; Chapter 2) and a recent three-year study, utilizing multi-locus DNA fingerprinting to ascertain paternity, reported an EPP rate of 7.7% in a population of Common Murres (Birkhead et al. 2001). A similar EPP rate of approximately 10% has been found for congeneric species, the Thick-billed Murre, *Uria lomvia* (Ibarguchi 1998). The current study uses microsatellites to determine the extent of EPP in the murres, a technique that has been used successfully for paternity testing in both passerine and non-passerine bird species (e.g., Swallows, *Hirunda rustica*; Primmer et al. 1995; Emus, *Dromaius novaehollandiae*; Taylor et al. 2000). The population of Common Murres analyzed in this study has been observed intensively since 1996 so that patterns of EPP can be related to behaviour and both breeding pair and individual histories. Parentage analyses were conducted on families sampled over four breeding seasons, 1996-1999. In-depth behavioural analysis are reported in Chapter 2 for each year for which paternity analysis was carried out, excluding 1996.

3.3 Methods

3.3.1 Field Methods

The study was carried out from May-July for the years 1996-1999 inclusive on Great Island (47°11'N, 52°49'W), Newfoundland. The breeding population of Common Murres on Great Island was estimated at 2800 pairs in the 1980s (Cairns et al. 1989), although recent observations suggest that the colony is expanding. In the current study,

the main study plot was a broad ledge (1.6 m X 2.5 m) on the southeastern side of the island, referred to as the DC site. A permanent wooden blind constructed adjacent to the plot allowed detailed behavioural observations to be made (see Chapter 2 for more detail about the site). By 1999, more than 50 adult murres were individually-marked and identifiable by unique colour band combinations, although the number of breeding sites monitored at the ledge increased from 18 in 1996 to 32 in 1999. Over the course of the study, 35 marked complete family units (chick and both parents) were examined to resolve if genetic parentage was consistent with social parentage as determined by behavioural observations. In 10 instances, chicks from the same families were observed and sampled in more than one year (6 families in three years, 4 families in two years). Thus, these family units were actually comprised of 19 *different* families from 1996-1999. The number of chicks that were examined for parentage in each year were: 6 (1996), 9 (1997), 10 (1998), and 10 (1999). Thus, a total of 35 chicks, 19 adult female parents, and 21 adult male putative parents (as male mate changes occurred for two families during the study) from the DC site were analyzed. In order to examine microsatellite variability, both intra- and inter-colony comparisons were made with samples taken from another two ledges on Great Island (total n = 21) and two sampling areas on Funk Island (49°45'N, 53°11'W; total n = 35), Newfoundland from 1995-1997.

For adult murres, a 1 ml blood sample was taken from the brachial artery and stored in Vacutainers™ containing no additives (red-topped clot tubes). This was to permit the extraction of serum used for hormonal analyses in another study. Serum was

removed from the samples and the remaining blood used for DNA extraction. To obtain murre chick DNA, chicks were caught when they were approximately 10 days old. A primary feather was extracted from each wing and placed in 70% ethanol.

3.3.2 DNA Procedures

Blood samples and chick feathers were kept at ambient temperature in the field and then at 4°C until DNA extraction procedures were carried out. Approximately 2-4 µl of whole blood were added to a buffer of 0.2M EDTA and 0.5% laurylsarcosine and incubated in 20mg ml of pronase E (Protease Type XIV, Sigma) at 37°C overnight. A series of three extractions with phenol, phenol – chloroform:isoamyl alcohol, and chloroform:isoamyl alcohol, respectively, were carried out, followed by precipitation with 95% ethanol at -20°C. Residual salts in the DNA were removed with a 70% ethanol wash, and the DNA was then left to air-dry or was dried in a vacuum flask. The DNA was resuspended in 40-400ml TE (pH 8.0). DNA samples were quantified using a fluorometer, and or were run out on an agarose gel to check for degradation.

To obtain chick DNA, the feather pulp of one primary feather was removed and minced finely before being placed in DNA extraction buffer. The rest of the extraction procedure was carried out as outlined above.

Four pairs of microsatellite primers developed for Common and Thick-billed Murres were obtained from G. Ibarguchi and V. Friesen (Queen's University, Kingston,

Canada): ulo12a12, ulo12a22, ulo14b29, and uaal-23 (see Ibarguchi et al. 2000 for details on microsatellite isolation and characterization). Sequences of the primers (5'→3') are: ulo 12a12, F: TCTACGATTCTATGATTCCACA, R: GATCTCTACCACAT-TCTCCCTA; ulo 12a22, F: TGAATGCAGTGTCAGTCAAG, R: TATAGGCTT-ATGCCAGAGAGAC; ulo 14b29, F: GTATTATGTTCCGGAAAA-CTGT, R: TACC-CCTATATACAAACCCAAG; ulo I-23, F: CCTGTGTTGAAAA-TAGAACAGA, R: TTTAGCTGGTGAAGTTAGTCAG. PCR protocols were modified from Ibarguchi, et al. (2000) and PCR reactions were conducted in a GeneAmp PCR system 9600 thermal-cycler (Perkin-Elmer). For each sample, approximately 50 ng of DNA were amplified in 25 µl reactions containing 0.1µM of reverse primer and 0.05 µM of forward primer (Queen's University CORTEC DNA Services Laboratories, Inc.), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 0.3 units of Tfl polymerase (Promega), 2.5 mM MgSO4 (Promega), and 0.3 µM ([^{32}P]-ATP (Amersham) end-labelled forward primer in 1X Tfl reaction buffer (Promega). General PCR conditions involved an initial denaturation at 94°C for 90s, followed by 34 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 45s. A final extension period of 72°C for 5 min occurred before samples were maintained at 4°C. PCR products were mixed with stop solution and were heated to 95°C for 5 min prior to being placed on ice and loaded into 6% polyacrylamide sequencing gel containing 19:1 acrylamide:bis-acrylamide, 7 M urea and 1X TBE buffer. Gels were run for 2-2.5 h at a constant power of 42W, placed in fixative containing methanol and acetic acid, dried for 2 h and then subjected to autoradiography at room temperature for 12-48 h. Each gel contained at

least one reference sample to ensure consistent scoring of alleles on all gels. Alleles were numbered arbitrarily.

3.3.3 Analysis of Genetic Parameters

Statistical analyses of population genetic parameters were carried out using the programs GENEPOP (Raymond and Rousset 1995*a*) and the allele frequency module of the paternity analysis program CERVUS (Marshall et al. 1998). To ascertain that microsatellite loci were not linked, genotypic linkage disequilibrium among loci was tested with GENEPOP, in which the Markov chain method provides an unbiased estimate of the probability value of obtaining the observed parameter. Exact tests for departure from Hardy-Weinberg and allelic and genotypic population analyses were also performed with GENEPOP. Such analyses are deemed suitable for microsatellite loci with many alleles per locus (Raymond and Rousset 1995*b*). The unbiased expected and observed single locus heterozygosities, H_{exp} and H_{obs}, were obtained using CERVUS, as was the estimated null allele frequency at each locus. If at least 25 individuals are typed for a given locus, CERVUS estimates the frequency of any null allele segregating at the locus, using an iterative algorithm based on the difference between observed and expected frequency of homozygotes. The paternity analysis module of CERVUS was run to check genotypic mismatches that had been identified by allele scoring of chicks and putative parents. The combined average exclusion probability, i.e., the average probability of excluding a single randomly chosen unrelated individual from parentage, both in the presence and in the absence of genetic information on the other parent was calculated for

all loci. The general assumption made was that the observed female parent was the genetic parent. The rare scenarios in which this assumption would not hold true include cases of adoption, for which there is some evidence in both the Common Murre (Harris et al. 2000; Wilson and Birkhead 2001) and the closely-related Thick-billed Murre (Gaston et al. 1995), or incorrect identity assignment of a chick or parent which might be due to either alloparenting or disturbance on the ledge during capture. Allele frequencies and population genetic parameters were obtained using adult Common Murres only in order to prevent bias from inclusion of related chicks. Locus ulo12a22 was only examined for paternity analysis.

3.4 Results

3.4.1 Amplification of Microsatellites

Amplification of DNA failed at one locus in four cases, and at two loci in one case, for the chicks in the 35 families examined. Thus, the parentage analysis was conducted on the 30 chicks for which the DNA of the chick and both of its putative parents amplified at a minimum of three loci excluding ulo 12a22 (i.e., amplification at loci uaal-23, ulo12a12, and ulo14b29 was required for inclusion in the analysis, see below). Of the five chicks that were excluded from the analysis, there was no evidence of EPP from the loci that amplified. In each case where chick DNA failed to amplify, the feather(s) used for DNA extraction were very small and the quantity of DNA recovered from the sample was low. Similarly, amplification failed at one or two loci for some samples taken from the two comparison ledges on Great Island and Funk Island.

However, for analysis of microsatellite diversity, all samples that amplified at a minimum of one locus were included. Amplification failures in these samples may be due to DNA degradation and the length of time from sampling to analysis.

3.4.2 Microsatellite Diversity

Analysis of linkage disequilibrium for each locus pair across populations (Fisher's method; GENEPOP) demonstrated no significant linkage between loci ($p>0.05$ for six tests). The polymorphism of the four microsatellite loci in Common Murres from this Newfoundland colony is generally lower than found for Pacific Common Murres (Ibarguchi et al., 2000) but is similar to that found in other Atlantic Common Murre colonies (M. Dumas, pers. comm.). Loci varied in their degree of polymorphism. Locus ulo14b29 was the most informative marker, with 17 alleles and an observed heterozygosity of 0.894 (Table 3-1). Locus ulo12a12 was also polymorphic, showing 9 alleles, including at least one null allele. The presence of a null allele at this locus, with an overall estimated frequency of 0.1502, is a probable cause of the discrepancy between the expected and observed heterozygosities at ulo12a12 (H_{exp}=0.764, H_{obs}=0.558, Table 3-1). Null alleles are not an uncommon occurrence at microsatellite loci and in paternity studies, the possible exclusion of putative male parents due to null alleles (and not EPP) must be considered (Pemberton et al. 1995). Locus ulo12a22 demonstrated the fewest alleles, with only 4 alleles present. Genic differentiation analyses suggested that the allelic distribution across populations was significantly different (Fisher's test with combined loci: $\chi^2_{(6)}$= 28.89, $p<0.00006$), suggesting that the populations may be

genetically subdivided; however, this result should be re-examined with larger sample sizes. Exact tests for departure from Hardy-Weinberg equilibrium (HWE) did not support the genic differentiation results in that deviation from HWE was significant for two loci only: ulo12a12 and ulo12a22. At locus ulo12 a12, this is likely due to the presence of a null allele, while the result at locus ulo12a22 may be due to the small number of individuals sampled.

Table 3-1. Polymorphism of four microsatellite loci in Common Murres sampled from two colonies in Newfoundland (Great Island and Funk Island; n = sample size: H_{exp} = expected heterozygosity; H_{obs} = observed heterozygosity).

Locus	n	H_{exp}	H_{obs}	No. alleles	Frequency of most common allele
ulo12a12	77	0.764	0.558	9 (including 1 - null allele)	0.3701
ulo12a22	35	0.529	0.714	4	0.5429
ulo14b29	85	0.878	0.894	17	0.1765
uaal-23	81	0.426	0.395	8	0.7469

3.4.3 Parental Exclusions

The total parentage exclusion power was calculated to be 0.812 (first parent) and 0.937 (second parent). Thus, the probability of excluding a random male as the parent given the genotypes of the female parent and chick was high, but was not absolute, i.e.,

there was approximately a 6% chance of *incorrectly* assigning paternity to the social father, when in fact, the chick was sired by an extra-pair male. For every chick, the genotype at each locus was compared with those of the putative parents, under the assumption that the putative female parent was the true biological parent. All but one mismatch that occurred between known female parents and offspring could be accounted for by the presence of a null allele at locus ulo12a12 as described below (Section 3.4.3.3), with the remaining maternal mismatch ascribed to adoption or alloparenting. Allele mismatches between chicks and putative male parents are summarized in Tables 3-2 and 3-3, with the rationale for the assignment of ambiguous cases to either EPP, adoption, alloparenting, misidentification of chick or parent, or the presence of a null allele and or mutation in the chick discussed below.

3.4.3.1 Extra-pair Paternity (EPP)

There were two cases in which offspring were conclusively shown to be genetically incompatible with the putative male parent (e.g., Figure 3-1; Tables 3-2 and 3-3). Both cases of EPP occurred in 1998. For chick 93, non-identifying exclusions with one parent occurred for both loci ulo14b29 and ulo12a12. Genotypes at locus ulo 12a22 confirmed that the mismatch was with the putative male parent. The "18" allele present in the offspring at locus ulo14b29 was presumably a mutation of the maternally-inherited "19" allele. There were no behavioural observations for pair 93 in the pre-laying season, as they were unmarked prior to the chick-rearing period.

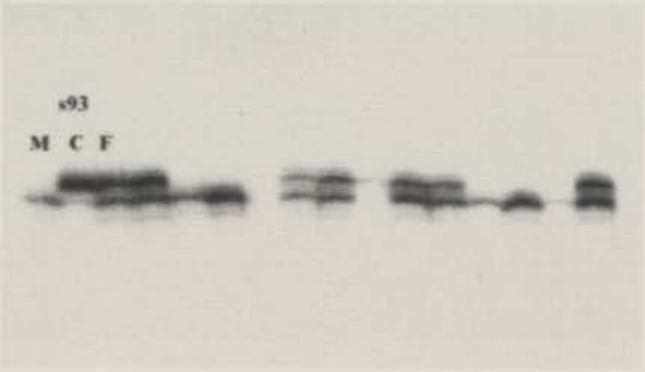

Figure 3-1. At microsatellite locus ulo12a22, the social male parent (M) in Family 93 possesses an allele which the chick (C) does not, confirming that the chick is the result of an EPF (F = female parent).

Table 3-2. The majority of chicks each year matched the putative male parent (n = 24); in cases where assignment of paternity was ambiguous (n = 6), the most likely explanation of the mismatch is identified.

YEAR	Cases of "Legitimate" Chicks	Ambiguous Cases			
		EPP	Adoption/ Alloparenting	Null allele/ mutation	Misidentified
1996	5 (chicks 6,10/20, 11/21, 30, 37)	---	---	---	1 (chick 99)
1997	6 (chicks 4, 5, 6, 17, 22, 84)	---	1 (chick 16)	---	---
1998	6 (chicks 4, 5, 12, 16, 17, "PAT")	3 (chicks 6, 84, 93)	---	1 (chick 24)	---
1999	7 (chicks 4, 5, 16, 17, 22,"PAT", 90)	---	---	---	---

Table 3-3. Genotypes for ambiguous chicks and their putative parents for the four microsatellite loci examined. "N"= null allele inferred; "M"= male; "F"= female; "no amp." indicates that the individual failed to amplify at this locus; "*" denotes where parent/chick mismatch(es) occurred.

Individual	Locus ulo 14b29	uaaI-23	ulo12a12	ulo12a22
chick 93	18, 23*	3, 7	11, 13*	7, 7*
93 M	23, 25	5, 7	3, 11	9, 9
93 F	19, 23	3, 7	7, 11	7, 9
chick 84	13, 23	7, 7*	6, N	9, 9
84 M	13, 25	9, 9	6, 6 (or 6, N)	7, 9
84 F	16, 23	7, 7	9, N	9, 9
chick 6	13, 19	1, 7*	7, 7 (or 7, N)	9, 9
6 M	13, 19	7, 7	7, 11	7, 9
6 F	19, 25	7, 7	N, N or no amp.	7, 9
chick 16	11, 21*	7, 7	8, 11*	7, 9
16 M	13, 19	7, 7	6, 7	7, 7
16 F	25, 27	5, 7	11, 11	9, 11
chick 99	21, 25	3, 7*	7, 7	7, 9
99 M	15, 25	7, 7	7, 13	7, 9
99 F	13, 21	7, 7	7, 11	7, 9
chick 24	19, 25	7, 7	8, N*	7, 7
24 M	15, 25	7, 7	N, N or no amp.	no amp.
24 F	19, 25	5, 7	7, 9	7, 9

In the other case, the chick in Family 84 was homozygous for allele "7", as was its female parent, while the putative male parent was homozygous for allele "9" at locus uaaI-23. Since there is no evidence of a null allele at this locus, this result most likely

excluded the male as the genetic parent. No mismatches occurred at other loci. Behaviourally, this pair was not seen to have engaged in any successful pair copulations (PCs), even though the male made pair copulation attempts which the female refused.

3.4.3.2 Mismatches due to EPP, Adoption/alloparenting, Chick Misidentification, or a Null Allele/mutation

There were four cases over the four years in which chicks failed to match either both parents or one parent whose identity could not be established. In one case, EPP is probable (chick 6), in another case (chick 16), adoption or alloparenting is probable, in the third case (chick 99), chick misidentification seems likely, and, in the final case (chick 24), mismatch of the chick and parents was arguably due to a null allele and a mutation at one locus.

1) **Chick 6**. The 1998 offspring of Family 6 possessed a rare allele at locus uaal-23 (allele "1"; frequency = 0.006) which was not present in either parent. It must be admitted that it is possible that this allele resulted from a mutation of one of the parental alleles "7" (i.e., both parents were homozygous at this locus). However, this mutation would have necessitated several repeat motif deletions in the chick, which might be unlikely (see Eisen 1999 for review of mutation in microsatellites). Allelic patterns at all other loci showed no genetic mismatch between the chick and its putative parents, so were not useful in identifying the source of the mismatch at uaal-23. However, in this species, it is improbable that the female parent would be unrelated to the chick, with the male parent simultaneously being the sire; if this was the case, the chick would have been sired by the

male with an extra-pair female and, then, the chick would have been adopted by the sire and his social partner. Such an event seems highly unlikely. The only other circumstance in which this could occur, the abandonment of the biological mother and her replacement with a new female (unrelated to the chick), was not a possibility in this case, as both 6M and 6F had been banded for several years prior to 1998. As well, based on behavioural observations, I am confident that the chick which was assigned to this family was indeed the social offspring. Observations following the chick's capture and marking indicated that the chick returned to pair 6's site and was fed there for the remainder of our observations. Thus, the chick was not misidentified during capture. In addition, this chick seemed to fledge late (at > 23 days of age), even though it was large and many other younger chicks had already departed. As murre chicks are accompanied to sea and attended to for several weeks after fledging by their male parent (Harris and Birkhead 1985), this observation *might* indicate that the male was "reluctant" to take the chick to sea due to uncertain paternity. Furthermore, there were no successful pair copulations observed in 1998, in spite of attempts by 6M (see Chapter 2). While 6F was not observed accepting EPCs in 1998, she did receive an EPC attempt from 5M that was believed to have been unsuccessful, i.e., did not result in cloacal contact. It is possible that this EPC attempt was successful, or was the prelude to another unobserved successful EPC between the pair. If either of these possibilities are true, 5M makes a likely paternal candidate for chick 6 in 1998, as the genotype of 5M at uaa I-23 is "2, 7", the chick's genotype at this locus is "1, 7", and it is possible that the "1" allele in the chick is a single repeat unit mutation of the male's "2" allele. Alternatively, 6F may have accepted an

unobserved EPC from a male possessing the "1" allele; however, none of the 21 males examined at this ledge showed this allele at locus uaa I-23. Thus, behavioural evidence of lack of pair copulations and a possible EPC, in conjunction with the non-identifying genetic exclusion, indicates that the most likely explanation for the mismatch in Family 6 is EPP.

2) **Chick 16**. At locus ulo14b29, the chick in 1997 had genotype "11, 21" while the putative parents had genotypes "25, 27" (female) and "13, 19" (male). Clearly, assuming correct identity of the chick and parents, there is no possibility that the adults are first-degree relatives of this chick. This is further supported by the chick's genotype at locus ulo12a12: the chick possessed alleles "8" and "11", while the putative female parent had genotype "11, 11" (or "11, N") and the male had genotype "6, 7". Thus, based on this locus, the female parent is not excluded, but the male could only be the genetic parent if a mutation occurred in the chick, altering the paternally-inherited "7" allele to the observed "8" allele by the addition of a repeat. However, in the context of the result from locus ulo14b29, this scenario seems unlikely. Two possible explanations for the mismatch between the chick and its putative parents exist: 1) the chick was adopted or was being alloparented when captured, or 2) the chick was misidentified as belonging to Family 16. We were unable to continue observations at the site after this chick was caught and banded and so are unable to be certain that this chick remained with these parents, although by this time most other chicks had fledged. It is possible that the few remaining unfledged and mobile chicks in the ledge intermingled during the capture and that the

chick that was at site 16 was not, in fact, the same chick that the parents were rearing. However, there was no doubt of this chick's identity among observers immediately prior to its capture, unlike the only other case of possible chick misidentification (chick 99, see below). Further support for the possibility of adoption was that several days prior to the capture of the chick from this site, observers noted that the chick at site 16 had disappeared and was presumed lost. However, on subsequent observation days there *was* a chick at that site, and it was presumed that the pair's chick had just wandered on the particular day that it was thought lost. This chick, which was then assumed to be the biological chick, may have been, in fact, a neighbour's chick that had either been adopted or was being alloparented at this site.

3) **Chick 99**. The 1996 chick from this family possessed an allele (allele "3") occurring at the relatively low frequency of 0.049 at locus uaaI-23. Neither parent possessed this allele. However, this was the only locus for which at least one parent was excluded; therefore, the identity of the mismatching parent could not be determined. Records of behavioural observations indicate that there was *a priori* uncertainty about this chick's identity before it was caught. The nest site of this family was not always clearly visible and it is possible that the chick labelled as belonging to this family was from a neighbouring site. In subsequent years, individuals at this nest site were not sampled due to our inability to conduct thorough behavioural observations there. Thus, the likely explanation for this mismatch is incorrect identification of the unmarked chick during capture.

4) **Chick 24**. Chick 24 in 1998 showed an allelic inheritance pattern at locus ulo 12a12 only that was not immediately consistent with either parent. An apparent mismatch with the female parent may be due to a *de novo* mutation at this locus: the genotype of the chick was homozygous for allele "8", the female parent was heterozygous with genotype "7, 9" and the putative male parent repeatedly failed to amplify at this locus only, suggesting that he was homozygous for the null allele ("N, N"). Thus, the true genotype of the chick may be "8, N" with the "8" allele being a mutation of one maternally-inherited allele.

3.4.3.3 Mismatches at Locus ulo12a12 due to a Null Allele

There were several mismatches between known female parents and chicks that could be ascribed to the presence of a null allele at locus ulo12a12 (Table 3-4). This null allele was easily detectable when examining family pedigrees: under the assumption that the female parent was indeed the true parent, there were twelve cases of mismatch between the offspring and female parent. One case, chick 24 in 1998, is described above (Section 4.4.3.2). In ten other cases (chicks 4 (1998), 5 (1997), 5 (1998), 5 (1999), 6 (1996), 6 (1997), 6 (1998), 37 (1996), 22 (1997), and 84 (1998)), allelic patterns from other loci support the probability that the most likely explanation was the incorrect classification of the offspring, female parent, or both as homozygotes due to the presence of a null allele. These ten female parent-offspring mismatches occurred only at locus ulo12a12: all other loci segregated as expected. The final case of female parent-chick

mismatch at this locus occurred in Family 16, as discussed above, and cannot be ascribed to the presence of a null allele in either individual.

Table 3-4. Genotypes of chicks and parents for cases of mother-chick mismatches due to a null allele ("N") present at ulo 12a12 at a frequency of approximately 15%. Chick 24 also demonstrated a de novo mutation.

Chick ID (Year)	Chick Genotype	Maternal Genotype	Paternal Genotype
chick 4 (98)	13, N	7, N	7, 13
chick 5 (97)	11, N	7, N	11, 11 (or 11, N)
chick 5 (98)	11, N	7, N	11, 11 (or 11, N)
chick 5 (99)	11, N	7, N	11, 11 (or 11, N)
chick 6 (96)	7, N	N, N	7, 11
chick 6 (97)	11, N	N, N	7, 11
chick 6 (98)	7, N	N, N	? (EPP)
chick 37 (96)	9, N	11, N	9, 9 (or 9, N)
chick 22 (97)	5, N	N, N	5, 5 (or 5, N)
chick 84 (98)	6, N	9, N	6, 6 (or 6, N)
chick 24 (98)	8, N	7, 9	N, N (or no amplification)

3.5 Discussion

The paternity of two chicks sampled in this study could not be definitively ascertained, as one chick was misidentified (s99 in 1996) and another (s16 in 1997) was likely being alloparented when it was captured. Thus, there were 7.1% (2/28) cases in which EPP could be definitively ascertained with four available microsatellites. In addition, there was one case of chick/parent genetic mismatch for which behavioural observations of no PCs corroborate that the parental mismatch was due to an extra-pair male, and not due to the chick mismatching the female parent. Five families were excluded from the paternity analysis for failure of the chick DNA to amplify at one or more of loci ulo12a12, ulo14b29, or uaaI-23. In these families, however, there was no indication of EPP at the loci which amplified. Thus, the overall EPP rate for this population of Common Murres over four breeding seasons is between 9.1 - 10.7%, depending on whether the number of chicks examined is considered to be 28 or 33. For a sample size of 28 chicks, the 95% confidence intervals for the EPP rate of 10.7% ranges from 2.3 - 27.9% (Rohlf and Sokal, 1981). This rate is consistent with that reported for other seabird species in which EPP has been found (e.g., Bukacinska et al. 1998; Graves et al. 1992) and is consistent with both the EPP rate found in another population of Common Murres, in a study using traditional multi-locus DNA fingerprinting (Birkhead et al. 2001), and the EPP rate detected in Thick-billed Murres, using the same set of microsatellites employed in the current study (Ibarguchi, 1998).

Because different chicks for some of the same families were examined in multiple years, this study analyzed 17 different sets of parents (and their chicks) over the study period. In 11/17 (approximately 65%) of these families, there was *never* an ambiguous assignment of chicks to putative male parents over the four years, i.e., chicks always matched the social male parent at all loci. In three of the remaining six families (i.e., 3/17 = 18%), there was evidence of EPP in one year only. In one of these families with EPP (pair 6), the chicks produced in the two years prior to the EPP chick matched the social male parent unambiguously. Similarly, in another case (pair 84), the chick produced in the year immediately before the EPP chick also matched the social male parent. In both of these families, no chick was produced after the EPP chick; pair 6 had subsequent hatching failure and pair 84 divorced in the following year. In the third family with EPP, pair 93, there was no previous information on the pair, including whether prior chicks had been produced, as this pair was caught and banded in the year that the EPP chick was hatched. Again, however, as seen in pair 84, this pair divorced in the year following the EPP chick. Thus, over the course of four years, there were no cases of EPP occurring in the same family in more than one year. Such inconsistency in which pairs produce EP offspring in different breeding seasons or, alternately, which males get cuckolded in different years, has been reported for Great Tits (Lubjuhn et al. 1999; 2001).

It is significant to note that, in this study, all confirmed cases of EPP were found in a single breeding season, 1998. If the study had been confined to this breeding season only, in the manner of several seabird EPP studies conducted to date (e.g., Graves et al. 1992; Austin et al. 1996; Hunter et al. 1992; Huyvaert et al. 2000), we might have

reported an EPP rate in this species three times higher than the rate that we obtained from our four breeding season-long investigation. While we concede that the sample size each year and, thus, the power to detect a case of EPP in any given year is low, the behavioural data for these murres indicated that there was significant among-year variation in the copulation activity of these murres. Specifically, in 1998, there was more PC activity relative to other years and the acceptance rate of PCs by females was lower in this year. Interestingly, the levels of EPC behaviour remained relatively constant from 1997-1999, and, with a few notable exceptions, different individuals were most often observed performing EPCs in different years (Chapter 2).

Of the 24 cases in which assignment of chick paternity was unambiguous, there were only six females that were observed to have performed EPCs. In all cases, females accepted from between two to nine PCs following their successful EPC(s). Thus, when EPCs were followed by PCs, there were no cases of EPP detected. This indicates that the behavioural acceptance of PCs by a female is an important factor in determining whether her chick will be sired by an EP male. Further support for this notion comes from two of the females for which an EPP was detected: both 6F and 84F were seen to refuse all PCs from their mates in the year that their EPP chicks were produced. An examination of behavioural data in order to determine whether there were any additional cases in which females were seen to have refused PCs from their mates (regardless of whether they accepted EPCs or not) indicated that there were: in 1999 only, two females (2F, 10F) were not observed to have participated in successful PCs, despite attempts by their mates. The chicks produced by these females were not analyzed for paternity, due to the fact that

blood samples for the entire family were not obtained. A further two females (7F, 11F) were also observed to have had no successful PCs in the Peak period of copulation (Chapter 2) in 1999, although no PC attempts by their mates were seen, either. In one case (11F), the female did have a successful EPC, possibly indicating an EPP outcome, although successful PCs were observed for this female prior to egg-laying. While the chicks from both females were examined, they were both eliminated from the final sample, as their DNA failed to amplify at two loci (ulo 12a12 and ulo 12a22). At the two amplifying loci, the chicks' genotypes were consistent with the social fathers being their sires.

If the refusal of PCs by a female is a predictor of EPP, then there were three cases of possible EPP (in addition to the three confirmed EPP cases) that occurred and which were either not sampled or were not detected due to primer amplification problems. If these cases had been sampled, successfully analyzed and were determined to be *bona fide* EPP, then the EPP rate in this study would have been 6/31 (19.4%). One caveat is that all of these potential EPP cases occurred in 1999, the year during which female attendance and PC rates were significantly lower than in other years (Chapter 2). It might be expected that, at least for the one case (7F) in which PC attempts were never seen, the pairs simply did not coordinate their activity well, i.e., the females did not actually refuse PCs, rather, they never received any observed attempts due to their erratic attendance in the ledge. Thus, this female, unlike the females (2F, 10F) which did refuse PC attempts (a rare event in 1999, the year of the highest PC success rates), might not be expected to have a chick sired by EP males if her later attendance facilitated the successful

performance of PCs. However, none were observed. This possibility is supported by the observation of 11F, for whom successful PCs were eventually recorded in 1999, when she and her mate were finally in the colony together. Thus, the maximum rate of EPP in this population, based on behavioural observations, is estimated at approximately 20%, although it is probable that this rate is somewhat inflated. Furthermore, it must be acknowledged that, based on the exclusion power of this set of microsatellites, it is statistically possible that approximately two cases of EPP were missed in a sample size of 28, i.e., two chicks might have been incorrectly identified as being sired by the social male parent. Thus, of the 28 analyzed cases, five (versus three) may have been EPP chicks; the overall EPP rate would then be 17.9% (versus 10.7%). Both of these possible EPP estimates fall within the 95% confidence interval of 2.3 - 27.9%, calculated in the basis of sample size. Indeed, based on the parameters of the multi-locus DNA fingerprinting technique used, Birkhead et al. (2001) calculated a confidence interval for EPP in Common Murres in the range of 1.1 - 19.3%. Our EPP rate, generated from paternity analyses with four microsatellites combined with extensive behavioural observations, concur with that obtained by Birkhead et al. (2001), possibly indicating that similar rates of EPP exist in different populations of Common Murres.

Many reasons have been posited to explain why birds should perform EPCs, including obtaining good genes, increasing genetic diversity among offspring, insuring fertility, and facilitating mate changes (reviewed in Birkhead and Møller 1992, 1998). The copulation histories of the murre pairs that experienced EPP suggest that EPP was related to mate changing in two instances and either seeking good genes or fertility

insurance in the other case (Chapter 2). Two of the breeding pairs in which EPP occurred were presumed young (families 84 and 93), as they had established new breeding sites during the course of the study. In both instances, the pairs divorced in the year following the EPP event. Thus, in both cases, mate changing in relatively newly-formed pairs seems to be related to EPP. In the final case, family 6, the pair was banded as breeding adults more than 10 years prior to the current study and, thus, was presumed to be older (a minimum of 15 years old in 1996). In the first two years of this study, the chick produced at this site genetically matched both male and female parents. Following the EPP occurrence in 1998, the eggs at this site repeatedly failed to hatch. At first glance, this suggests reproductive senescence in the pair over the course of the study and, perhaps, points to the female seeking EPCs as a means of fertility insurance in 1998. However, the non-hatching of this pair's eggs may be more likely due to the non-viability of the chick embryos produced from 1999-2001. Evidence that this pair may be closely related supports this notion (see Chapter 4), as chick embryo mortality is often related to close inbreeding (Kempanears et al. 1996).

While Birkhead et al. (2001) reported an EPP rate similar to that found in the current study in a population of Common Murres from Skømer Island, U.K. they did not report the temporal distribution of EPP incidents. Thus, it is unknown whether EPP rates differed significantly among the three years of their study. As well, it is unknown whether factors leading to EPP in their study were similar to those presented here, as behavioural data for the individuals that were analyzed were not reported.

The clustering of all confirmed cases of EPP in one breeding season in the present study, along with corroborating behavioural evidence, suggests that for long-lived species, such as the Common Murre, examination of the frequency of EPP should occur in the context of long-term studies. It is feasible that individuals of species with reproductive longevity can alter their reproductive strategies over time in response to social, environmental, or individual factors. Such a response could temporally alter the incidence of EPP in a given population. As well, based on the breeding pairs which experienced EPP in this study, it is possible that short-term studies may be at greater risk of biasing the EPP outcome if a disproportionate number of very young or old breeders are sampled, as such individuals might be expected to experience higher EPP rates. Indeed, other studies have reported that reproductive behaviours (e.g., parental investment, divorce) change significantly with age, experience, or length of the pair bond (Forslund and Pärt 1995; Heg et al. 1993). Certainly there is evidence that, in some species, older males obtain a disproportionate number of EPCs (e.g., Brook and Kemp 2001); EPP rates might be expected to vary with such demographic factors as well.

Equally important in studies which examine EPP is the inclusion of behavioural observation that can determine whether any parent-chick genetic mismatch is likely due to EPP or other factors, such as adoption. In a species, such as the Common Murre, that nests in large colonial groups, some degree of misidentification is inevitable if individuals are not marked, as is the case with chicks prior to their capture and sampling. Thus, behavioural observation should be an integral component of EPP studies in birds whenever possible.

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Chapter 4
Genetic Relatedness Among Common Murres in Two Newfoundland Colonies

4.1 Abstract

A minimum of two microsatellite loci were used to estimate Hamilton's (1964) coefficient of relatedness (R) for Common Murres in two Newfoundland colonies. Within each colony, murres were examined from different areas or ledges in which genetic substructuring on the basis of extended kin might exist. The pairwise relatedness of known parent-chick pairs, full-siblings, half-siblings, unrelated dyads and social mates from one ledge, as well as the relatedness of females performing extra-pair copulations and their extra-pair males was also estimated. Average R values indicated that this sample of murres, as a whole, was unrelated, as were individuals within each colony overall. However, when examined by ledge/area, R values were more variable, with some ledges showing high average relatedness. Due to unequal sample sizes between ledges, interpreting differences in R values is problematic. On average, known parent-chick and full-sibling relationships produced R values that were near the theoretical expected value of R = 0.50, although there was a large amount of variation in the R values for pairs of individuals. The average pairwise coefficient of relatedness for social mates, half-siblings and unrelated dyads could not be distinguished from each other. Females and their extra-pair mates were not significantly more or less related than social mates overall. These findings are discussed in relation to previously published work on the congeneric Thick-billed Murre and are placed in the context of philopatry, kin selection, and inbreeding in seabirds.

4.2 Introduction

Molecular markers have permitted us to determine the genetic relatedness of individuals present within geographic locales, which, in turn, has informed our knowledge of sex-biased breeding dispersal, inbreeding, and the presence of kin groups within the breeding populations of several different species (e.g., mound-building mice, *Mus spicilegus*, Garza et al. 1997; European wild rabbits, *Oryctolagus cuniculus*, Surridge et al. 1999; brown long-eared bats, *Plecotus auritus*, Burland et al. 2001). These molecular techniques now permit estimation of Hamilton's (1964) coefficient of relatedness (R), in the absence of detailed family pedigree information obtained by exhaustive behavioural observations and or capture-recapture methods (Palsbøll 1999). As coefficients of relatedness gauge the genetic similarity of focal individuals relative to a reference population, the genetic structure both within and among social groups can be elucidated with this information (Ross 2001).

The presence of relatives within a social group has potential effects on the evolution of behaviours exhibited by individuals within the group (e.g., altruism via kin selection; Hamilton 1964; Axelrod and Hamilton 1981; Queller 1992). As well, if mates are closely related to each other, the possible negative genetic consequences of inbreeding might be expected to become manifest within the group (e.g., inbreeding depression; Shields 1982, 1993; Greenwood 1987; Rowley et al. 1993). Interestingly, there is recent evidence for local kin structuring within some species in the *absence* of significant inbreeding, indicating that philopatry and inbreeding do not necessarily co-occur (e.g.,

Red Grouse, *Lagopus lagopus scoticus*, Piertney et al. 1999; greater horseshoe bats, *Rhinolophus ferrumequinum*, Rossiter et al. 2000).

Primarily based on banding studies, it has been largely accepted that many seabirds are philopatric, i.e., they return to their natal colonies to breed as adults (e.g., Manx Shearwater, *Puffinus puffinus*, Perrins et al. 1973; Atlantic Puffins, *Fratercula arctica*, Harris 1983; Black-legged Kittiwakes, *Rissa tridactyla*, Coulson and Nève de Mévergnies 1992; see review in Greenwood and Harvey 1982). Philopatry in some seabirds has been more recently documented with molecular techniques as well (e.g., Cory's Shearwater, *Calonectris diomedea*, Rabouam et al. 1998; 2000). High site fidelity across years is also exhibited by many seabird species, including murres (*Uria* spp.; Gaston and Jones 1998). In fact, both Common (*U. aalge*) and Thick-billed Murres (*U. lomvia*) exhibit high levels of philopatry, both to their natal colonies and, to a lesser extent, to their natal subcolony or ledge (Swann and Ramsay 1983; Hudson 1985; Gaston et al. 1994; Halley et al. 1995). On Coates Island, Thick-billed Murres on their first breeding attempt established nest sites within a median distance of 2.6 metres from their own hatching sites (Steiner and Gaston 2000). While some murres recruit to their natal ledges, there is significant dispersal from colonies as well, i.e., it is likely that large numbers of new breeders recruit to non-natal colonies (Harris et al. 1996). Indeed, the finding that Thick-billed Murres showed no evidence of macrogeographic genetic variation among five Atlantic colonies (Birt-Friesen et al. 1992) suggests that dispersal of murres to non-natal colonies is relatively common. Indeed, in expanding or long-established colonies for which there is intense competition for nest sites, philopatry to

natal areas may not be possible. Instead, new ledges of breeding birds may be established within colonies when recruits cannot easily return to natal ledges.

In spite of the absence of macrogeographic population differentiation among Atlantic colonies of Thick-billed Murres, Friesen et al. (1996*a*) reported significant microgeographic differentiation within one Norwegian colony, Hornøya. Specifically, based on allozyme and mitochondrial DNA (mtDNA) analyses, there was significant substructuring among ledges, evidenced by a mean coefficient of relatedness (R) value within ledges of ~0.10. Thus, there was evidence that the Hornøya ledges contained extended kin (about the level of first-degree cousins, R = 0.125) in the absence of population substructuring among colonies. Interestingly, similar results were obtained for Bulgarian mound-building mice (Garza et al. 1997). Kin-association was high within specific mounds (analogous to murre subcolonies), but there was minimal genetic differentiation between the fields in which the mounds were located (Garza et al. 1997). Indeed, for mammals, at least, it has been suggested that micro- and macro-geographic differentiation are independent processes (Pope 1992).

We do not know yet whether such a pattern of microgeographic difference in the absence of among-colony differences is common in murres. In contrast to Friesen et al. (1996*a*), Ibarguchi (1998) found only weak evidence for microgeographic differentiation within Thick-billed Murre ledges using both cytochrome b markers and microsatellites. Thus, it is possible that kin associations are not ubiquitous in murres; rather, such

relatedness patterns may reflect colony- or ledge-specific parameters such as recent population bottlenecks (Ibarguchi 1998), or age structure.

In contrast to the lack of population differentiation in the Thick-billed Murre, the congeneric Common Murre shows a cline in the genotype frequencies of cytochrome b among five Atlantic colonies (Friesen et al 1996*b*). This genetic cline is similar to a cline in the incidence of bridling (the presence of an auricular eye ring) within the Atlantic colonies (Friesen et al. 1996*b*). The authors suggested that this cline was the result of secondary contact between two refugial populations from the Pleistocene glaciations, *not* the result of current restricted gene flow, and, thus, it may disappear over evolutionary time.

Two of the colonies sampled by Friesen et al. (1996*b*) were sampled in the current study: Great Island (Witless Bay) and Funk Island. I examined evidence for or against local kin structuring within three ledges on Great Island and within one ledge and one large area on Funk Island on the basis of R values obtained with some of the same microsatellites developed and used by Ibarguchi (1998; see also Ibarguchi et al. 2000). Due to sampling regimes, I expected to find no evidence of kin structure in at least one sampled area (i.e., "Funk Centre", the main subcolony of Common Murres on Funk Island where birds were caught as they flew into the area), but was uncertain if population substructuring would exist on other ledges. One ledge, the subcolony studied in Chapters 2 and 3, was examined in detail, as it contained individuals for which I had family pedigree information beginning in 1996.

4.3 Methods

4.3.1 Samples

Common Murres from Great Island and Funk Island, Newfoundland were captured, banded, and blood samples taken as described in Chapters 2 and 3.

4.3.2 Calculation of Relatedness

The relatedness coefficient, R (Hamilton 1964), was estimated using individuals' genotypes from three autosomal microsatellite loci (ulo 14b29, ulo 12a22 and uaa I-23; described in Chapter 3) analyzed with the software program RELATEDNESS 5.0.8 (Queller and Goodnight 1989). This program generates R values ranging from –1.0 to 1.0, with a negative value indicating that individuals are less closely related than expected for an average pair based on population or group allele frequencies (for example, in cases where individuals are from two different population sources). The formula used for calculating R is:

$$\frac{\sum_{x}\sum_{k}\sum_{l}(P_{y}-P^{*})}{\sum_{x}\sum_{k}\sum_{l}(P_{x}-P^{*})}$$

where x indexes the numbers of individuals examined, k indexes the microsatellite loci, l indexes the allelic position, P_x is the frequency within the current x individual of the allele at x's locus k and allelic position l, P_y is the frequency of that same allele in the individuals to which x's relatedness is measured, and P^* is the frequency of the allele in

the population at large, with putative relatives of *x* excluded (Goodnight and Queller 1989; Goodnight 1999).

Using this program, average coefficients of relatedness were calculated for all individuals sampled, for individuals within the two colonies separately, and for individuals within discrete ledges or areas of the two colonies. Average R values were also calculated for a sub-sample of known males and females from the DC Ledge (i.e., the site on Great Island where behavioural observations were carried out; Chapter 2). In order to remove potential bias in the R values introduced by including close relatives in the sample (Queller and Goodnight 1989), average R values were calculated only from the genotypes of adults (i.e., chicks were excluded), and, with the exception of the overall R for all murres sampled, allele frequencies corrected by ledge area. This allele frequency bias correction removes only those individuals belonging to the same ledge area as the current X individual (for which R is being calculated) from contributing to the calculation of the overall allele frequencies, P*, and thereby prevents the allele frequencies of (potentially) related individuals from underestimating the relatedness coefficient. No allele frequency bias correction was applied to the calculation of the overall R estimation for the entire sample (n = 94), as the sample was deemed large enough that no single set of relatives would be likely to make a significant contribution to the overall allele frequencies (Goodnight 1999). Standard errors were obtained by jackknifing over loci which involves dropping out each locus in turn, calculating a new statistic for each reduced data set, and, once this process is completed (over *n* loci), calculating a standard error from the entire set of values obtained (as per Sokal and Rohlf 1981; Queller and

Goodnight 1989). Pairwise estimates of relatedness were obtained for all individuals, including chicks, on the DC Ledge, and between randomly generated adult dyads in which one member was from Great Island and one was from Funk Island. As large standard errors are expected with such pairwise estimates (Queller and Goodnight 1989), they were not reported for each individual pair. However, to ensure that the R values obtained were close to the expected values for known relatives, 30 mother-chick pairs (expected R = 0.5), 28 father-chick pairs (expected R = 0.5), 11 full sibling pairs (expected R = 0.5), and 6 half-sibling pairs (expected R = 0.25) for which individuals amplified at two or more loci were examined. Cases of ambiguous parent-chick mismatches were excluded for the parent(s) with which the chick's genotype was inconsistent (e.g., in cases of extra-pair paternity, EPP, the pairwise calculation of R between the male parent and the chick was excluded from the calculation of the average father-chick R value; see Chapter 3). The relatedness coefficient for 30 pairs of unrelated murres (i.e., one individual from each of the two colonies) was also examined to test the validity of the calculated R values (expected R = ~0.0, or negative if the source populations have significantly different allele frequencies at these loci). Finally, the estimated pairwise relatedness coefficients of 1) social mates (n = 20), 2) females that participated in extra-pair copulations (EPCs) and their social mates (n = 8; a sub-sample of (1)), and 3) females that participated in EPCs and their EP mates (n = 5) were examined. Local allele frequencies for the DC Ledge were calculated from the genotypes of adults only and supplied to the RELATEDNESS 5.0.8 program for the calculation of pairwise R values.

The presence of a null allele at a high frequency (approximately 15%) at one of the four microsatellite loci used for the parentage analyses reported in Chapter 3 is problematic for accurately determining relatedness (Pemberton et al. 1995; Primmer et al. 1996). As several cases of mother-chick mismatches occurred due to the non-amplification of at least one (null) allele in which mothers and/or chicks presented as homozygotes (Section 3.4.3.3, Chapter 3), it was deemed unreasonable to use this locus for calculation of relatedness. While it was possible to score genotypes at locus 12a12 fairly accurately for individuals from the DC Ledge for which family pedigree analysis permitted the detection of a null allele(s), it was not possible to determine for other homozygotes whose chick did not show a mismatch whether they, in fact, carried a null allele. Thus, the overall incidence of one or more null alleles in this sample would have been underestimated if an attempt had been made to include locus ulo 12a12 in the analysis of DC Ledge individuals. As well, there was no way of accurately determining the presence of a null allele in individuals from areas other than the DC Ledge for which there was no family pedigree information. Eliminating locus 12a12 from the current analysis reduced the possible number of loci used to calculate R to three loci for individuals from the DC Ledge, and to two loci for all other individuals (i.e., locus ulo 12a22 was examined for individuals in the parentage analysis from the DC Ledge only).

4.4 Results

4.4.1 Average Relatedness Estimates

The overall relatedness coefficient calculated for all murres was –0.014 (s.e. = 0.003, n = 94; Table 4-1), i.e., taken as a whole, the birds were unrelated. Mean relatedness coefficients were also fairly low for individuals within the colonies, but were much more variable for individuals within areas or ledges, ranging from –0.026 to 0.173, although the effect of different sample sizes on different ledges must be considered (Table 4-1). Within the DC Ledge of Great Island, interestingly, the R value among males was not different than that for females (males: R = -0.080 (s.e. = 0.070); females: R = 0.019 (s.e. = 0.083)). Due to small sample sizes of some ledges, statistical differences in R values within ledges were not reported as their meaning would be uncertain; i.e., with a larger sample size, a lower overall relatedness value might be expected (due to the possible presence of different kin groups) than for a smaller sample size (which might include only one kin group). However, it is interesting to note that the ledge on Great Island for which individuals have the lowest overall relatedness value is "2L" (R = -0.026; s.e. = 0.057; n = 14), an area which has been expanding since 1996. As expected, a similar value was obtained for individuals from Funk Centre (R = -0.010; s.e. = 0.186; n = 11), the main flat-topped subcolony of Funk Island at which birds were caught with nets as they flew into the area. For these Funk Centre individuals, there was no possibility of identifying from which part of the central murre area these birds originated.

Table 4-1. Mean R values ± standard error among all murres in the data set, among individuals within the two colonies of Great Island and Funk Island, among individual within specific ledges or areas of both colonies, and among males and females within the DC Ledge of Great Island*.

Sample	N	R (= standard error)
All murres	94	-0.014 (= 0.003)
Great Island	61	0.046 (= 0.095)
DC Ledge	40	0.090 (= 0.115)
2L Ledge	14	-0.026 (= 0.057)
BLL Ledge	7	0.137 (= 0.075)
Funk Island	33	0.058 (= 0.128)
Funk Ledge	22	0.173 (= 0.059)
Funk Centre	11	-0.010 (= 0.186)
Males (DC Ledge)*	21	-0.080 (= 0.070)
Females (DC Ledge)*	19	0.019 (= 0.083)

* Calculation of sexed individuals from the DC Ledge only used 3 loci.

4.4.2 Pairwise Relatedness Estimates

Comparison of the mean pairwise R values for unrelated dyads from different colonies, social mates, mother-chick pairs, father-chick pairs, full siblings, and half-siblings indicated that first-degree relatives were effectively detected, on average, with

the microsatellites used in the study ($F_{(5, 119)} = 17.0$, $p<0.01$; One-way ANOVA). The estimated relatedness coefficients for social mates and half-siblings were not significantly different than that for unrelated dyads (Table 4-2), while the R values for mother-chick pairs, father-chick pairs, and full siblings were significantly different from those of unrelated dyads, social mates, and half-siblings ($p<0.03$, Tukey's HSD post-hoc test). Interestingly, the range of pairwise relatedness coefficients was large; for example, for mother-chick pairs, the R values ranged from –0.123 to 0.835.

There were eight social pairs in which the female accepted EPCs at least once from 1997-2000 and for whom a relatedness coefficient between the pair members could be calculated. However, since there was no genetic information on several males which attempted EPCs (i.e., they were unbanded or their DNA was not analyzed), there were only five female-EP male dyads for which a R value could be estimated. There was no significant difference between the mean relatedness coefficients of social pairs in which the female accepted EPCs ($R = 0.009$, s.e. = 0.112; $n = 8$) and "female-EP male" pairs ($R = 0.0004$, s.e. = 0.139; $n = 5$; $t_{(11)} = 0.05$, ns), nor was there any difference in the relatedness values for these social pairs in which females accepted EPCs (R reported above) versus social pairs in which females did not accept EPCs ($R = 0.010$, s.e. = 0.094; $n = 12$; $t_{(18)} = -0.008$, ns). In the two cases for which the genetic relationship between females' social mates and these females' EP partners could be analyzed, the R values were very low ($R = -0.416$, $R = -0.429$). This could indicate that these females chose EP males that were genetically dissimilar to their social mates.

Table 4-2. Average pairwise R values ± standard error and theoretical expected R values for unrelated dyads, social mates, mother-chick pairs, father-chick pairs, full siblings, and half siblings.

Sample	N	R (± standard error)	95% Confidence Interval of Mean	Expected R
Unrelated dyads*	30	-0.041 (± 0.066)	-0.177 to 0.094	0.00
Social mates	20	-0.010 (± 0.070)	-0.137 to 0.156	?
Mother-chick pairs	30	0.419 (± 0.051)	0.315 to 0.523	0.50
Father-chick pairs	28	0.519 (± 0.046)	0.424 to 0.613	0.50
Full siblings	11	0.494 (± 0.068)	0.342 to 0.646	0.50
Half siblings	6	0.007 (± 0.137)	-0.345 to 0.359	0.25

*R values for unrelated dyads were based on two loci (ulo 14b29, uaa 1-23) using the population allele frequencies from the entire sample, corrected by ledge/area, while all other pairwise comparisons used three loci (ulo 14b29, uaa 1-23, ulo 12a22) and local allele frequencies of the DC Ledge.

While the range of pairwise relatedness estimates within both mother-chick and father-chick pairs was large, the individuals in the cases of parent-chick mismatches described in Chapter 3 all showed R values that were lower than both the expected R of 0.50 (under the assumption that the parent was the genetic parent) and the average R values calculated for parents and chicks in this study (mother-chicks, R = 0.419; father-chicks, R = 0.519; Table 4-3).

Table 4-3. Pairwise relatedness coefficients for parents and "mismatched" chicks are lower than both the expected theoretical R (0.5) and the calculated average pairwise R values for first-degree relatives (father-chicks, R = 0.519; mother-chicks, R = 0.419).

Chick	Reason for mismatch	Pairwise R value
93 (1998)	EPP	father-chick: -0.566
84 (1998)	EPP	father-chick: -0.113
6 (1998)	EPP	father-chick: 0.361*
16 (1997)	Adoption/alloparenting	father-chick: 0.187
		mother-chick: -0.497
99 (1996)	Misidentification	father-chick: -0.010
		mother-chick: 0.067

* The relatively high value between 6M and this "EPP" chick may be due to the high relatedness coefficient between 6M and 6F (R = 0.566).

4.5 Discussion

4.5.1 Utility of Microsatellites for Relatedness Analysis

The set of microsatellites employed in the relatedness analysis of these Common Murres was effective at discriminating between first degree relatives (i.e., R = 0.50), *on average*, and unrelated individuals. However, there was a high degree of variability among the estimated R values between pairs for all known categories of first- and second-degree relatives. In fact, half-siblings (i.e., second-degree relatives) could not be

distinguished from unrelated dyads, likely due to the relatively low number of loci used and the heterozygosity/ allelic distribution of these loci (e.g., one locus, ulo 12a22, had only four alleles; Goodnight and Queller 1999; Blouin et al. 1996). The probability of misclassifying dyads of either first or second degree relatives as being unrelated was high; thus, while it would be desirable to manufacture a pedigree for adult neighbours within the DC Ledge, such a derivation would likely be inaccurate. The addition of one or more loci to this set of microsatellites would lower the probability of misclassification of relatives and permit a detailed examination of the relatedness coefficient of individuals within ledges.

Although the interpretation of single between-pair relatedness values must be treated with caution, the cases of mismatches between chicks and putative parents due to EPP, adoption or alloparenting, or mistaken identity of individuals generally showed the pattern of estimated R values being lower than both those that would be expected theoretically and the mean values that were calculated for known parents and chicks. The only exception to this was the EPP of chick 6 (1998); in this case, the relatedness coefficient between the putative father and chick was 0.361. This relatively high value for a chick and his (allegedly) non-genetically related father might be due to the high pairwise R value between 6F and 6M (R = 0.566). Indeed, the possibility that pair 6 is inbreeding at the level of first-degree relatives provides a *post-hoc* explanation for the repeated failure of egg-hatching at this site from 1999-2001. In each of these years, a chick was not hatched despite the facts that pair copulations and extra-pair copulations by the female were observed, and that the egg was incubated constantly. Lifjeld et al. (1994)

and Birkhead et al. (1995) suggested that non-hatching of eggs is often due to the mortality of chick embryos and not egg infertility. In several passerine species, there does appear to be a relationship between hatching failure and close inbreeding (Bensch et al. 1994; Kempenaers et al. 1996; 1999). Thus, if 6M and 6F are first-degree relatives, their hatching failures might be explained due to the non-viability of the chicks (for example, if lethal mutations were inherited by the chick). However, the possibility that either the male and/or female have become infertile since 1999 cannot be completely excluded, as the pair was at least 18 years old by 1999. The likelihood of reproductive senescence at this age in Common Murres is unknown; certainly, individual Common Murres and individuals of the closely-related Thick-billed Murre species have been known to breed successfully for more than 20 years (Gaston and Jones 1998; A.J. Gaston, pers. comm.), although a detailed examination of the relationship between advanced age and reproductive success has not been reported for the Alcids.

4.5.2 Relatedness among Social Mates

There was no indication that social mates in the DC Ledge were closely related to each other overall, although it appears that some matings between close relatives did occur. Thus, close inbreeding within Common Murre ledges can probably be found, but likely occurs at rather low frequencies under normal circumstances, i.e., in populations not under high mortality pressure due to hunting, food scarcity, or other stressors (Friesen et al. 1996*a*; Ibarguchi 1998). Assuming that there is a genetic component to survivorship under such harsh conditions, the individuals likely to survive might be more closely

related to each other than the subpopulation would be under normal conditions. Interestingly, in two other seabird species, Cory's Shearwater and Wilson's Storm Petrel (*Oceanites oceanicus*), there is evidence that social mates are more closely related to each other than nonmates and, in both these species, there has been no evidence of EPP found (Rabouam et al. 2000; Quillfeldt et al. 2001, respectively).

It has been suggested for some species that females perform EPCs to obtain indirect genetic benefits for offspring, including increased genetic diversity of offspring (reviewed Birkhead, 1998). Indeed, a meta-analysis of avian genetic diversity and the EPP rates of certain species showed that higher EPP rates were found in populations with greater diversity (Petrie et al. 1998), a pattern that might be expected if the females choose EP males on the basis of their genetic dissimilarity to themselves or their mates (Bensch et al. 1994). However, in an analysis of Blue Tits (*Parus caeruleus*) and Great Tits (*Parus major*), Kempenaers et al. (1996) found no evidence that the genetic similarity between females and their EP males was lower than that between females and their social partners. A similar finding was found in the current study for Common Murres (albeit the sample sizes of these groups were small): the average R value between females and EP males was not significantly different than that between the females and their social mates. As well, there was no evidence that the females which accepted EPCs were any more or less closely related to their social mates than females which refused EPCs. Thus, there is no support for the hypothesis that female murres perform EPCs to obtain increased genetic variability of offspring. However, in two instances, EP males were highly unrelated to the social mates of the females which accepted their EPC

attempts. This could indicate that genetic dissimilarity between a female's mate and a potential EP partner is a factor in determining which males a female will accept EPCs from. Certainly, a much larger sample size is required before this possibility can be evaluated.

4.5.3 Kin structure in Murre Colonies?

Using cytochrome b markers for mitochondrial DNA (mtDNA) and polymorphic allozyme loci, Friesen et al. (1996*a*) detected microgeographic differentiation of Thick-billed Murres on ledges in Hornøya, Norway. There, ledges of murres showed an average relatedness coefficient of between 0.10 and 0.13. Thus, the murres were related at the level of first cousins, although there was no evidence of population differentiation on a macrogeographic scale (i.e., between colonies) for this species (Friesen 1992; Birt-Friesen et al. 1996). One possible explanation of this pattern in the Hornøya colony may be a severe food restriction and subsequent population crash which occurred several years prior to the sampling (Friesen et al. 1996*a*). Thus, such a pattern of local genetic structure might not be expected in other colonies where such factors are non-existant.

In a similar study on Thick-billed Murres from Coates Island, NWT, Canada, Ibarguchi (1998) examined the genetic variation of a relatively large number of individuals (N = 290) from different ledges using both cytochrome b and three microsatellite loci, two of which (ulo 14b29, uaa I-23) were used in the current study of

relatedness[1]. Her results for relatedness coefficients using microsatellites were similar to those reported here: the range of R estimates for individuals within ledges was very large and, while the overall "within ledge" average R values were lower than expected based on the Hornøya study, there were individual cases of dyads within ledges that were highly related (i.e., R> 0.125), as well as individuals that were highly *un*related (i.e. R between -0.29 and -0.20). Relatedness estimates obtained for cytochrome b were generally higher than those for the microsatellites and some ledges showed that groups of close kin (R > 0.10; first cousins or grandparents-grandoffspring) were caught within the same area. Overall, Ibarguchi (1998) concluded that there was a weak but consistent trend for more related birds to be found on ledges than expected by chance alone, although there were also many birds on these ledges that were unrelated. This suggests that the global "within ledge" estimates of relatedness could be lower than actual values due to fine-scale kin structure within a ledge. If small groups or "pockets" of kin were found within a single ledge, each possessing their own unique allele frequencies, the average relatedness value for individuals on the ledge would be low in spite of actual ledge structuring (Queller & Goodnight 1989).

The current relatedness analysis on Common Murres also provides limited evidence for substructuring of ledges on the basis of kin groups. In the DC Ledge of Great Island, for which there was limited pedigree information from a behavioural and parentage study (Chapters 2 and 3), the overall relatedness of adults was moderate but

[1] The remaining microsatellite locus developed and used by G. Ibarguchi was ulo 12a12, for which there was no evidence of a null allele in the Thick-billed Murre. This locus was excluded from the relatedness analyses for the presence of a null allele at high frequency in the congeneric Common Murre.

was associated with a fairly large standard error (R = 0.090 ± 0.115). Similarly, the range of pairwise estimates of R values between social mates was large (-0.640 to 0.609), i.e., some individuals appeared highly related while others were highly unrelated. Interestingly, adults on a discrete ledge of Funk Island (Funk Ledge; n = 22) had a relatively high relatedness coefficient, with a fairly small standard error measurement (R = 0.173 ± 0.059). Thus, individuals on Funk Ledge appeared to be related at a level above first cousins, although there is currently no information concerning the social relationships between these individuals. However, as these murres were captured during the late chick-fledging periods of 1996, it is likely that many are females who were attending the ledge after successful fledging of their chicks (Wanless and Harris 1986). Murres from the BLL Ledge on Great Island also had relatively high coefficients of relatedness (R = 0.137 ± 0.075), although the sample size for this ledge is small (n = 7) and this R value may be unreliable. However, as this ledge was sampled by leaning over a cliff-top and catching any adults on narrow ledges that were within reach of the noose pole, the high relatedness value might provide further support for the notion that kin could cluster on some ledges (i.e., a "pocket" of kin might have been sampled on BLL). It should be noted that for the DC Ledge, there is no clear evidence of small clusters of kin being spatially distributed among the ledge. When R values were superimposed on a map of the DC Ledge, there was no obvious pattern of more related individuals nesting near each other. Interestingly, the lowest R values for any ledge or area examined in this study was for the 2L Ledge and the Funk Centre area. The 2L Ledge is located to the south and slightly above the main study ledge and has expanded considerably since 1996. As there appears to be little or no room left for newcomers on the main (DC) ledge, or on the face

of the cliff from which the DC Ledge extends, it is possible that 2L Ledge is an area to which new breeders are coming (Harris et al. 1996). If other areas (e.g., natal ledges) in which there is restricted space are the preferred breeding locations of the new recruits, it is possible that these individuals on 2L Ledge were completely unrelated. Similarly, the Funk Centre area is in the main subcolony of Common Murres breeding on Funk Island, and contains on the order of 50,000 breeding pairs (W. Montevecchi, pers. comm.). Since these birds were caught as they flew into the subcolony, it is highly likely that they were from different parts of this area, and were likely to be unrelated. The physical attributes of ledges might also be related to whether philopatry or substructuring by kin groups is likely. Individuals on a wider ledge (like the DC Ledge) might be more unrelated to each other than individuals breeding on a narrow ledge (such as the Funk Ledge), if the latter, who are arguable more constrained physically by space, preferentially permit kin to join the ledge.

4.5.4 Philopatry and Kin Selection in Common Murres

Within the subsample of sexed individuals from the DC Ledge, females were slighly (albeit nonsignificantly) more related to other females than males were to each other. This is a finding consistent with Ibarguchi's (1998) finding using microsatellites for a subset of Thick-billed Murres. However, when Ibarguchi sexed all the birds within the colony, it was clear that, based on cytochrome b markers, the pattern of male relatedness, but not female relatedness, within the colony was significantly non-random

(Ibarguchi, pers. comm.). In fact, male genotype clumping was significant between the east and west sides of "Fox Gully", a natural gully in the main colony cliff that separates many ledges. No such patterns were detected with microsatellites, suggesting that there may be a male-biased mutation rate for these polymorphic microsatellites in particular, and for hypervariable markers in general (Ibarguchi et al. 2001). Interestingly, there was high female relatedness on some ledges, but the overall pattern of relatedness from cytochrome b markers suggested that male Thick-billed Murres might be the more strongly philopatric sex, at least in the Coate's Island colony (Ibarguchi, pers. comm.). There was no evidence of a sex difference in philopatry of Common Murres on the DC Ledge of Great Island based on the current microsatellite analysis. It should be noted that in a recent review of avian dipsersal, Clarke et al. (1997) pointed out that the existing literature suggests that it is inappropriate to consider a sex bias in dispersal to be a species constant.

Given that both philopatry and dispersal occur in murre and other seabird species, it is interesting to speculate on the factors that determine which individuals return to their natal colonies and which ones disperse (Coulson and Nève de Mévergnies 1992). As immatures often make pre-breeding visitations to their natal colony (Halley et al 1996), it is likely that other immatures prospect at other colonies and/or the non-natal areas of their own colony (Lyngs 1993; Swann and Ramsay 1983). The possibility is intriguing that there are certain factors associated with an area (e.g., food availability or predation rates) and/or particular social interactions which occur during prospecting (e.g., affiliative behaviours with kin or EPCs with a ledge resident(s)) that either increase or decrease the

likelihood of that area ultimately becoming chosen as an individual's breeding site. As well, one might predict that the degree of relatedness of an individual to others in the natal ledge or colony could influence its return. For example, would the offspring of one parent which is breeding on its natal ledge and one parent which was recruited from another colony be more or less likely to attempt to breed in the natal area than an offspring of parents which were both breeding in their natal ledge? Determining the answers to such questions would be difficult; although if such answers were found, they would most certainly increase our understanding of philopatry, dispersal, and kin selection in seabirds.

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Chapter 5
Summary

The performance of EPCs by many socially-monogamous avian species, including the subject of this thesis, the Common Murre, has been well documented in recent years (Birkhead and Møller 1998). However, the overall incidence of EPP in *at least* two broad taxonomic classifications- passerines vs. non-passerines- varies considerably, with most non-passerine species exhibiting either no EPP or low rates of EPP even in the presence of significant amounts of EPC behaviour (EPP rates discussed in Fleischer 1996; Petrie and Kempenaers 1998). One factor which makes determining the underlying causes of among-taxa differences in EPP rates difficult is the fact that many EPP studies do not include relevant analyses of EPC behaviour. Rather, the rates of EPC behaviour are often inferred from EPP rates (Petrie and Kempenaers 1998). Thus, for many species, it is difficult, if not impossible, to directly compare EPC behaviour with EPP outcome. The meaning of EPP rates in species *outside* of the context of their EPC behaviour is vague and uncertain. If there is an interest in truly understanding this ubiquitous occurrence of EPC behaviour in socially-monogamous birds, versus in simply cataloguing frequency of EPP in various species, it is imperative that long-term behavioural observations of copulation and social behaviour be linked to paternity outcome analyses. Several critical questions then arise: If EPCs are performed and do not (often) result in EPP, how has this behaviour evolved and why do individuals engage in EPCs? Such answers are not easy to come by, but will provide the foundation for our understanding of the evolution of male and female alternative mating strategies.

In some species, particularly non-passerines, there is evidence that females obtain direct benefits from EPCs that include fertility insurance, acquisition of nutrients, paternal care, and facilitation of a partner change (reviewed in Birkhead 1998). However, according to Birkhead (1998), females of most species do not show any such direct benefits of accepting EPCs, so it has been assumed that these females must perform EPCs for indirect genetic benefits. However, the theory that females perform EPCs to gain such benefits for their offspring, either "good genes" or increased genetic variability appears to be losing ground (Kempenaers et al. 1999; Lubjuhn et al. 1999, 2001). In Great Tits, for example, in which approximately 36% of broods investigated contained extra-pair young, none of the predictions of the "good genes" theory of EPP were upheld (Lubjuhn et al. 1999). For example, if EP males were chosen for "good genes", one would expect both these males and their offspring to have increased rates of survival but, in fact, none was found (Lubjuhn et al. 1999). As well, males that were cuckolded in one year were no more likely to be cuckolded in the following year than males who were not cuckolded at all (Lubjuhn et al. 1999). One possibility in this species is that the availability of high quality males is different for female Great Tits in different years (Lubjuhn et al. 1999). At a minimum, such results indicate that factors other than the genetic quality of males play a role in determining female participation in EPCs.

If there is growing evidence that the "good genes" theory of EPC behaviour is inadequate to explain EPCs in species for which a significant number of extra-pair young are produced, what about species, such as seabirds, in which EPC behaviour apparently leads to very few cases of EPP? There are at least two possible explanations for such

observations: 1) in such species, the male social mate is always favoured through sperm competition or female choice, and/or 2) females perform EPCs for reasons other than obtaining genetic benefits.

5.1 Male Sperm Competition, Female Choice, or Both?

By definition, sperm competition implies that the "best" male wins the opportunity to sire a female's offspring. There are several connotations for the meaning of "best" in this situation: it is possible that the male with the most genetically compatible sperm is "best", or that the male with the largest volume of viable sperm is "best", or that the male who times his copulation properly prior to ovulation is the "best" (Birkhead and Møller 1998). However, is the "best" male winning the fertilization because of sperm competition or because of female choice? In the case of the male with the most compatible sperm obtaining fertilization, it has been argued that such an outcome could be the result of cryptic female choice within the female's reproductive tract (Keller and Reeves 1995). However, if either the "last male" advantage or "representational sperm hypothesis" explains sperm competition of birds (Birkhead 1998), then the most critical component of determining paternity must be the female's choice of who to mate with, when to mate with him, and/or how many times to mate with him. In Common Murres, females *do* largely control the pre-copulatory behavioural act of either accepting or refusing both EPCs and PCs. For the majority of females that accept EPCs, EPFs do not result, as these females typically accept more PCs from their social mates following EPCs. Thus, for a species or population in which the social male is always mated with

last, or is mated with more frequently than any EP males, the expected paternity outcome would be low or no EPP.

The directionality of the relationship between pair bond instability and EPC behaviour is difficult to determine. The relative infrequency with which EPP occurs in this species makes it hard to assess the circumstances under which females may perform EPCs for the facultative purpose of obtaining an EPF. However, it may be relevant that in the two cases of EPP for which we observed their pre-laying behaviour, PC acceptance behaviour of the female was altered in the year that the EPP chick was produced. This suggests that when EPCs are performed by females for genetic benefits, it might be the alteration of PC behaviour which is critical in determining the paternity outcome.

Of course, the decision of females to engage in or refuse EPCs is not necessarily conscious at the level of the individual female. If there are significant costs to a female involved with a mate detecting EPP (Mauck et al. 1999), then any behavioural rule which would lower the probability of EPP, such as "(under most circumstances) copulate last or more with your mate", might be expected to have evolved. Assuming that a female is mated to an average or above average partner (i.e., as a pair they experience average reproductive success or better), performing this strategy over the long-run should be successful for the female, as her risk of potential costs associated with EPP are minimized and her reproductive success is at least average. However, the indirect benefits of EPP to females might outweigh potential costs; for example, females with a mutant strategy of *failing* to copulate last or more with their mates *might* end up with a superior EP

offspring. Assuming heritability of EP behavioural tendencies in offspring (in sons, male attractiveness which leads to female acceptance of EPCs; in daughters, female acceptance of EPCs), the tendency to perform EPCs might be expected to spread. Thus, even if females did not perform EPCs for genetic benefits *per se*, accepting some EPCs, along with the concomitant failure to accept pair copulations or to accept them in a timely fashion, could enhance the spread of EPC behaviour if there are associated genetic benefits.

EPC behaviour, with or without modified PC behaviour, could be facultative for individual females. Given their individual social circumstances, such as having a low quality partner or poor reproductive success, it could be adaptive for females to either accept or reject EPC attempts from males. If accruing genetic benefits were important, this would involve females' assessments of the potential of the EP male as a sire of young relative to that of their own mates, or, if other direct benefits were obtained from EPCs (such as facilitating mate change), it could involve assessment of the EP male as a future mate. Thus, individual females could (and likely do) exercise individual discrimination in whether to accept EPCs, who to accept them from, and when to accept them.

5.2 EPCs for Non-genetic Benefits?

It is likely that, in some species, EPC behaviour in females has evolved for reasons other than accruing indirect genetic benefits (see above). In Common Murres, EPCs may function simply to ensure that the female maintains some sperm in the event

that her mate is either infertile or absent from the colony when she is present, or it could have a social function in terms of facilitating relationships between individuals that would be useful if a mate change is imminent. The latter notion is supported by the acceptance of EPCs by females under two circumstances: 1) a small number of females accepted EPCs only prior to their first reunion with their mate in the colony, and 2) for females, the likelihood that they would accept EPC behaviour was increased when they were widowed, divorced, or *in a social pair bond that was later broken over the course of the study*.

Some females were seen to have accepted EPCs only when their mates had not yet arrived in the colony (or at least before the pair had been reunited). After the pair was reunited, no more EPCs were accepted by these females. The function of such isolated instances of EPC behaviour seems clear: females were behaviourally insuring against the non-return of their mates, i.e., in the event that this occurred, a potential new social relationship had already been forged by the female's acceptance of a male's EPC attempts. The females which showed this pattern of EPC behaviour all had high reproductive success with their social mates over the course of this study.

Not surprisingly, females who were singled either by non-return/death of a mate or through divorce also performed EPCs with paired and unknown males, presumably in an attempt to form a new social bond. However, the fact that female EPC behaviour was also seen in females, but not typically in males, who were to *become* divorced (up until and including the 2001 field season), suggests that the "pre-divorce" acceptance of EPCs

by these females was to facilitate future mate change. However, could the EPC behaviour of these females have caused their future divorces via male retaliation? This seems unlikely, as three of the five divorced females who performed EPCs were the choosers of the divorce, i.e., they left their mates and breeding sites (Ens et al. 1993). Of these three choosers, two were never observed to accept EPCs once their new pair bonds had been formed.

The fact that EPCs could have evolved in certain species for non-genetic benefits does not preclude the possibility that, over time, genetic benefits of EPCs have accrued (Jennions and Petrie 2000). However, in this study, there is little evidence that EPP existed in isolation from social pair bond disruption. Specifically, two of the three females with EPP in this study divorced their social mates in the year following the EPP occurrence. In one case the female was the chooser of the divorce, while in the other case, the female was labelled victim as her mate left their site for another female. In one case (the female victim), no successful pair copulations were observed at all during the pre-laying season of the year in which the EPP chick was produced, despite copulation attempts by the male. Similarly, there were no successful PCs observed in the third pair in which EPP was detected; interestingly, this pair has remained together, and the female has accepted EPCs in subsequent years, although the pair has had no reproductive success since the EPP chick was raised. The fact that this pair appears to be related on the order of siblings ($R = 0.566$) might explain both their poor chick hatching record (Bensch et al. 1994 ; Kempenaers et al. 1996), as well as their failure to divorce. Although highly speculative, it is possible that this male might be tolerant of EPP since he would still be

related to EPP chicks) by virtue of his genetic relationship with the female (i.e., he could be at least an "uncle" to any EPP chick).

The relatively low frequency of both EPCs and EPP and the relationship between EPC behaviour and pair bond instability suggest that the *primary* function of EPC behaviour in female Common Murres is *not* to obtain indirect genetic benefits. While this possibility is not excluded, and some females did obtain EPFs, other direct benefits of EPCs, particularly, that they function to facilitate future mate change, are more likely.

5.3 Genetic Structuring of Colonies

If the acceptance of EPCs by females is primarily due to the possibility of obtaining indirect genetic benefits from males, one would expect to find the highest rates of EPP in species with the highest amounts of genetic variability. In fact, such a finding had been reported: the greater the genetic variability in populations of various species, the greater the EPP rate (Petrie et al. 1998). This finding may have direct implications for EPC and EPP rates of genetically structured colonies of seabirds (Friesen et al. 1996). If cliff ledges or colonies have low amounts of genetic variability by virtue of high relatedness of individuals, low rates of EPP might be expected. In fact, in two recent studies, colonial seabirds with relatively low genetic variability and relatively high coefficients of relatedness among social mates, at least, were shown to have no EPP at all (Rabouam et al. 2000; Quillfeldt et al. 2001).

If it is accepted that there is a positive relationship between EPP and genetic variability, then in colonies which are substructured by kin, low rates of EPC behaviour and EPP outcomes might be expected, since most available EP mates would largely not be genetically dissimilar from females' social mates. However, if ledges are loose amalgamations of kin and non-kin, then some EPC behaviour and EPP would be expected, with EP males being genetically dissimilar to the social mates of the females which accepted their EPC attempts. In the DC Ledge of Common Murres, the overall coefficient of relatedness showed that the inhabitants of the ledge were relatively unrelated. Even among social mates, though, there was a large range of relatedness coefficients, suggesting that some adults were closely related to each other while others were unrelated. Interestingly, females which accepted EPCs were no more or less closely related to their mates than females which refused EPCs, nor were they more or less closely related to their EP mates than they were to their social mates. However, for the two cases in which I could compare the genetic relationship between an EP male and the social mate of the female that the EP male copulated with, the males were highly unrelated, i.e., they were genetically dissimilar. This might suggest that these females chose EP males based on factors (morphological or behavioural?) related to their genetic dissimilarity with the females' social mates. Obviously, due to such a small number of cases, such a possibility requires further exploration in this species.

The evidence for genetic substructuring within Common Murre colonies is weak, but the relatedness patterns obtained generally conformed to those found in a similar study of Thick-billed Murres (Ibarguchi 1998). Specifically, while individuals in the main

study ledge on Great Island were unrelated overall, there were individuals which were closely related to each other. Two other ledges, one in each colony, showed moderate overall relatedness, while individuals from one large area in Funk Island and individuals from a newly expanding ledge on Great Island showed low relatedness. While the set of microsatellite markers used for this analysis were not without their problems (discussed below), they were effective, on average, at differentiating first-degree relatives from unrelated individuals. Thus, the average relatedness values obtained within ledges/areas are probably reliable and reflect the overall pattern of relatedness within the ledges, at least for those individuals that were sampled.

5.4 Limitiations of Microsatellites Used

While microsatellites can be powerful markers that can resolve paternity, for example, with near 100% accuracy (Parker et al. 1998), the set of four primers used in this thesis were less effective than was desired. This was likely due to the particular heterozygosities of loci in this population of Common Murres as well as to the low number of loci amplified (Blouin et al. 1996). In addition, amplification of DNA at one locus (ulo12a12) demonstrated the presence of at least one null allele at a significant frequency; this eliminated the use of this locus for the RELATEDNESS 5.0.8 analysis employed to examine genetic structuring within and among colonies of Common Murres. As well, since only complete families were analyzed with all four loci, many individuals were analyzed with only three loci.

The set of microsatellites used in this thesis consisted of imperfect dinucleotide-repeats that were highly polymorphic in both Thick-billed and Common Murres (Ibarguchi 1998; Ibarguchi et al. 2000). In the current sample, mutation rates were likely high at locus ulo 12a12, as a new allele was observed in one chick (of a total sample size of 30). This places the estimated mutation rate at 1.67×10^{-2} per gamete, which is near the high end of mutation rates for avian microsatellites (3.6×10^{-2}: Primmer et al. 1996). In the Thick-billed Murre, Ibarguchi (1998) obtained high mutation rates for both this locus and for locus uaa I-23, although, because she lacked pre-laying season behavioural data, she was unable to clearly differentiate between possible mutations and incidents of EPP in all cases. Likely due to differences in murre population sampling regimes, or to smaller sample sizes in the current study, there were significantly fewer alleles recorded at two loci (ulo 14b29, uaa I-23) for the Common Murre from Newfoundland than for the sample reported by Ibarguchi et al. (2000). This could suggest that alleles exist in other Common Murre populations (e.g., the Pacific populations sampled by Ibarguchi et al. 2000) at these loci that are absent or rare in the Atlantic colonies of Great Island and Funk Island, Newfoundland.

5.5 Future Directions

There are some simple improvements that would strengthen the existing genetic data reported in this thesis. First, the flanking primers for locus ulo 12a12, at which there is evidence of a high frequency null allele(s), could be redesigned in an attempt to amplify the null allele(s). This has been successfully done for cross-amplification of

microsatellites in bear species (*Ursus spp.*: Paetkau and Strobeck 1995). Secondly, all samples that were analyzed with only three loci could be augmented by analysis with the fourth microsatellite (locus ulo 12a22), thereby improving the resolution of the analysis of genetic relatedness within and among colonies. Thirdly, there is now available a fifth polymorphic microsatellite, uaa5-8, developed in Common Murres (Ibarguchi et al. 2000). Augmenting both the paternity data and the microgeographic analysis with this additional microsatellite would likely improve the power of both studies. Lastly, there are chick-parent samples from both the 2000 and 2001 field seasons that need to be analyzed to increase the sample size of complete families in the EPP study.

Aside from the technical improvements that seem possible, the greatest improvement to this study of copulation behaviour, EPP, and relatedness among individuals in the DC Ledge of Great Island would involve increasing the sample size. Due to the limited number of individuals in the ledge, sample size could only be increased in one of two ways: 1) increased catching of unmarked individuals, and 2) increased number of breeding seasons during which marked individuals are both observed and sampled.

It is difficult to increase the sample size of the individuals that currently reside in the ledge, as many of them are now marked, and those which have not yet been caught could not be caught (i.e., they are out of reach or they have continuously eluded the noose pole). Due to the fact that catching is only performed during early pre-laying and late chick-rearing periods when disturbance impacts are presumed to be the lowest, there are

occasionally new individuals that arrive in the ledge, do something significant (such as perform an EPC), but cannot be not caught. There is probably little that can be done in such situations, as catching at times other than those described could jeopardize the murres breeding attempts. As long as the Great Island project continues, we can only hope to catch and band as many unmarked individuals as possible.

It is my hope that the study of reproductive behaviour and paternity of Common Murres continues at the DC site on Great Island for many more years. Given the unique proximity to the birds that this location offers, as well as the accumulation of behavioural and genetic data for resident murres beginning in 1996, this study site could provide continuing insight into the evolution of EPC behaviour that so requires the combination of in-depth, long-term behavioural studies with genetic analyses.

5.6 References

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