

MATING BEHAVIOUR AND PATERNITY OF SOCIALLY MONOGAMOUS
SEMIPALMATED PLOVERS
CHARADRIUS SEMIPALMATUS BREEDING IN THE SUB-ARCTIC

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Mating Behaviour and Paternity of Socially Monogamous
Semipalmated Plovers *Charadrius semipalmatus* Breeding in the
Sub-Arctic

Yuri Zharikov

The extra-pair copulation (EPC) patterns and the extent of extra-pair paternity (EPP) are known for few monogamous Arctic-breeding birds. Here, I present results of a two-year study of Semipalmated Plovers *Charadrius semipalmatus* which involved behavioural observations of mating interactions between individuals in the field and parentage testing using multilocus DNA fingerprinting. It is argued that in the conditions of the Arctic where the breeding season is generally short and a large time/energy investment of both parents in incubation and brood rearing is crucial for their survival, the costs of engaging in extra-pair activities will out-weigh possible benefits to both males and females. Thus, both EPC and EPP rates will be low. In agreement with my predictions the observed frequencies of EPCs and EPP were low with an EPC rate of 7.1%. Extra-pair fertilizations occurred in 4.1% of families (1/24) resulting in an extra-pair paternity rate of 4.7% (4/85). My results generally agree with conclusions of studies suggesting that short breeding season/high breeding synchrony and open nesting habitat will lead to generally low extra-pair paternity rates in species with pronounced mate-guarding.

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In many avian species male and female individuals establish strong associations during breeding that lead to social monogamy. Until fairly recently up to 90% of all bird species were thought to maintain seasonally or life-long monogamous pair bonds (Lack 1968). This paradigm, however, has been all but completely overturned during the last ten years as parentage attributed to outsiders has been discovered in broods of many species (e.g. Westneat 1987, 1993a, b, Sheldon & Burke 1994).

The phenomenon of extra-pair parentage (parentage attributed to an individual other than the one providing parental care) has received overwhelming attention in behavioural ecology after development of the method of DNA fingerprinting exploiting the hypervariable nature of polymorphic tandem repeat loci (Jeffreys et al. 1985a, b). This method allowed for a relatively unambiguous determination of parentage in full family groups. It has proven to be an exceptionally useful tool in determining true mating strategies and realized reproductive success of individuals in many species across different taxa, reproductive systems and ecological conditions. The proportion of extra-parental young found in different species and populations has been revealed to range from 0 to

60% (see Westneat et al. 1990, Birkhead & Møller 1992 for reviews).

Trivers (1972) first suggested that extra-pair copulations (EPCs) (copulations between a member of a pair and an individual other than a social mate, Westneat et al. 1990) may occur when individuals engage in a mixed reproductive strategy. That is, an individual maintains a social bond with, and provides parental assistance to a permanent partner, yet seeks copulations outside of the pair. Extra-pair copulations and extra-pair paternity (EPP) resulting from these copulations are now accepted as widespread phenomena among socially monogamous birds (see Westneat et al. 1990, Birkhead & Møller 1992 for reviews) to the extent that their presence is probably as common as their absence. Despite or perhaps due to the widespread occurrence of EPCs and EPP, a great deal of variation in frequencies of these occurrences has been found both at the interspecific (see Gyllenstein et al. 1990, Dunn et al. 1994a, Millar et al. 1994, Sundberg & Dixon 1996) and intraspecific levels (e.g. Great Tit *Parus major*: Blakey 1994, Verboven & Mateman 1997; or Pied Flycatcher *Ficedula hypoleuca*: Altalo et al. 1987, Lifjeld et al. 1991, Gelter & Tegelström 1992, Rätti et al. 1995). Origins of this variation are still poorly understood and attempts are being

made to elucidate the combination of ethological and ecological factors responsible for it (e.g. Slagsvold & Lifjeld 1997).

There are three general mechanisms through which extra-pair parentage can arise. The first, and by far the most common is through extra-pair copulations. The second mechanism, termed intra-specific brood parasitism (ISBP) or 'egg-dumping' is also fairly wide-spread but much less so than EPCs (Petrie & Møller 1991, Birkhead & Møller 1992). Here, a foreign conspecific female lays an egg in the nest so that both members of the pair raise a genetic stranger. The third mechanism through which extra-pair parentage can arise is termed 'quasi-parasitism' (Wrege & Emlen 1987, McKittrick 1990); it is found in few avian species. This phenomenon results from a female other than the in-pair female copulating with the in-pair male and then laying an egg in his nest. This creates a situation where the in-pair female raises genetically unrelated young.

Ecological conditions are thought to have an important role in EPC/EPP rates in bird populations. Specifically, breeding density and synchrony (Westneat et al. 1990) as well as habitat features (Sherman & Morton 1988, Westneat et al. 1990) may be of particular importance in influencing the frequency of extra-pair fertilization events. Results of

studies on different species and different populations of the same species indicated that in some situations there was a positive association between breeding density and EPP rates (e.g. Hoi & Hoi-Leitner 1997, Verboven & Mateman 1997) while in other studies no such relationships could be found (e.g. Dunn et al. 1994b, Rätti et al. 1995). While the possible effect of breeding density of EPP rates seems rather clear, that is higher breeding density provides a greater opportunity for individuals to interact and thus engage in extra-pair activities (Møller & Birkhead 1993a, but also see Westneat & Sherman 1997), the role of breeding synchrony is more controversial (Dunn et al. 1994b, Weatherhead 1997). One hypothesis attributed particular importance to the role of males in controlling EPCs (Westneat et al. 1990). It predicted that high breeding synchrony should drive the EPP rate down as males would be busy guarding their mates and copulating with them. A more recent publication (Stutchbury & Morton 1995), however, accounted for the role of females in (or control of) EPCs and used a more general definition of breeding synchrony (i.e., high synchrony in temperate regions versus low synchrony in the tropical zone). This study provided support for a positive correlation between the incidence of EPP and breeding synchrony. It suggested that female

songbirds receive a greater opportunity to assess the quality of potential extra-pair mates and thus choose possible extra-pair partners of higher quality than the males they are paired with when more conspecifics are breeding synchronously.

Generally, researchers agree on a number of hypothesized costs and benefits which EPCs and EPP provide to both males and females. Males sire additional offspring without investing energy and resources in them but they may have to trade off mate-guarding and therefore may risk cuckoldry (Whittingham & Lifjeld 1995, Yezerinac *et al.* 1995) and/or harassment of their own mates by other males seeking EPCs. Females are thought to receive several indirect benefits from EPCs including 'good genes' (Birkhead & Møller 1992) or good sperm (Yasui 1997) from better quality extra-pair males. The existence of these hypothesized benefits was subsequently supported by some experimental data (Kempnaers *et al.* 1992, Hasselquist *et al.* 1996, Sundberg & Dixon 1996, Kempnaers *et al.* 1997). Older males are generally more successful in achieving extra-pair paternity (Wagner *et al.* 1996, Weatherhead 1997) than their younger conspecifics. Females may also gain genetic diversity of the offspring which may make the latter more adaptable to changing environmental conditions as well

as assure clutch fertilization if the pair male's sperm are unviable (Westneat et al. 1990). Participation by females in extra-pair activities may also be a way of minimizing the possibility of inbreeding (Brooker et al. 1990) or outbreeding depression (Rätti et al. 1995). The suggested drawbacks of females' involvement in extra-pair events may include their physical punishment by in-pair males and/or withdrawal of parental care (Møller & Birkhead 1993b, Dixon et al. 1994). Strong supportive evidence for the latter is lacking. In birds, parents almost universally cannot distinguish between their own and foster offspring even if the young result from interspecific brood parasitism (Kempnaers & Sheldon 1996 and references therein).

In a recent paper on extra-pair fertilizations in the Water Pipit *Anthus spinoletta* Reyer et al. (1997) argued that extra-pair fertilizations resulting from chance events arising through the temporal and spatial distribution of breeding pairs rather than females' search for genetic and/or phenotypic benefits offer a better explanation for occurrence of extra-pair activities. This hypothesis appears to provide a reasonable basis for observed trends and patterns of extra-pair events in species in which a well defined mixed reproductive strategy is not realized, i.e., in situations where extra-pair activities do not appear to

confer clear benefits to either sex and the percentage of extra-parental young is low.

Whatever the evolutionary reasons for females engaging in extra-pair copulations, males should find ways to safeguard their paternity, thus creating a situation of sexual conflict. To prevent their mates from being fertilized by others, males employ two strategies which are not necessarily mutually exclusive (Dickinson & Leonard 1996): frequent copulations and/or mate guarding.

Frequent in-pair copulations are thought to be used when close mate-guarding is impossible due to certain ecological or ethological constraints, such as the need to defend breeding sites (Lifjeld et al. 1993 and references therein) or (semi)colonial breeding when guarding against numerous conspecifics would be energetically costly (Hunter et al. 1992). In some species these copulations appear to be relatively ineffective paternity guards (Dunn & Robertson 1993, Lifjeld et al. 1993). This may be because extra-pair copulations can be disproportionately more successful than in-pair copulations (IPCs) due to, for example, the higher sperm count from males involved in EPCs (Birkhead et al. 1995). In other species in which EPCs are known to occur, in-pair copulations alone seem to be an adequate means of paternity defense (Hunter et al. 1992).

Species with intensive mate-guarding are typically expected to have lower EPP rates than species primarily relying on frequent in-pair copulations as a means of paternity assurance because the former typically occur at lower breeding densities. Anatomically, frequently copulating species are expected to have larger relative testes size (Møller & Briskie 1995) than species that mate-guard which results from their need to retaliate frequently when the female becomes inseminated by extra-pair males.

Most paternity studies conducted to date have focussed on small songbirds (Order Passeriformes) that typically occupy visually occluded breeding environments and often have low annual survival and subsequent return rates. Relatively few representatives of other orders have been examined (e.g. Lawless et al. 1997 (Strigiformes), Piper et al. 1997 (Gaviiformes)). In addition, most studies have been conducted in temperate conditions where breeding seasons are fairly long and thus the temporal constraint on breeding individuals is weaker than in more northern climates. No study to date has addressed paternity patterns of a socially monogamous Arctic-breeding shorebird using molecular genetics techniques. In fact only a handful of papers have examined paternity in any shorebird (e.g. Oring

et al. 1992, Heg et al. 1993, Owens et al. 1995, Lanctot et al. 1997) (Appendix 1).

The purpose of this study was to investigate the occurrence and frequency of extra-pair copulations and paternity in the Semipalmated Plover *Charadrius semipalmatus* - a small shorebird nesting in coastal and inland tundra and wetlands from Alaska and northern Canada south to the Maritime Provinces, James Bay, Great Slave Lake, British Columbia, and the Queen Charlotte Islands (AOU Check-list 1983). Other than records of numbers of fledged young, the Semipalmated Plover's reproductive behaviour has not been studied in sufficient detail to provide information on individual realized reproductive success, as determined through DNA fingerprinting in many other species (e.g. Gibbs et al. 1990).

Study species

The Semipalmated Plover is a territorial, sometimes gregarious species with well defined sexual dimorphism in plumage colouration and wing and tarsus lengths (Cramp and Simmons 1983, Teather & Nol 1997). Females are noticeably duller and slightly larger than males. The survival of clutches and young in the Semipalmated Plover depends on biparental care to the extent that incubation and brooding would appear nearly impossible without the presence of both

members of a pair as these responsibilities are divided equally between them (Sullivan Blanken & Nol 1998, pers. obs.). The species is considered socially monogamous with males typically paired with one female and vice versa. Pairs are formed for one or more breeding seasons (Flynn 1997).

Semipalmated Plovers nest in areas of open habitat (shoreline, coastal tundra) where visual contact between individuals is possible over a considerable distance. Two breeding densities are found in the species in the study area: solitary nesting pairs and loose nesting aggregations (Rippin Armstrong & Nol 1993) or neighbourhoods. The number of pairs of the species nesting in the area is sufficiently high to facilitate behavioural observations and allow for interactions among breeding birds. Multi- (i.e. feeding and nesting) as well as single-purpose (i.e. only nesting) breeding territories are established by males and usually defended by both members of a pair. Females are closely guarded by males throughout the pre-laying period and copulations are infrequent. Breeding synchrony is high whereas renesting attempts are uncommon (Nol *et al.* 1997). The sex ratio in the area is close to 1:1 (Flynn 1997). "Floaters", consisting of either failed breeders or non-breeding individuals, are either very few or they appear in

the area only when most of the resident pairs have already nested. Territorial males without a mate are also rare.

Predictions

High breeding synchrony, open breeding habitat, a short pre-laying period and intensive mate guarding by males may lead to low EPC and subsequent EPP rates (Birkhead & Biggins 1987, Birkhead & Møller 1992, Slagsvold & Lifjeld 1997, Westneat et al. 1990). These circumstances, as well as the very short breeding season typical for the Arctic/Sub-Arctic climatic zone, suggest that the level of EPP in the Semipalmated Plover population should be low to absent. There is, however, anecdotal evidence of EPCs in the bird (L. Flynn, pers. comm.) and thus the probability of occurrence of extra-pair paternity may be relatively high although EPC rates are not always a good predictor of EPP in birds (Birkhead & Møller 1995, Dunn & Lifjeld 1994).

If EPP is detected in the Semipalmated Plover population it is expected that cuckolded males will be younger, less familiar with the area and unable to provide their mates with an adequate level of mate guarding. In addition, certain ecological conditions of nesting may play a role too; e.g. males using separate territories for nesting and feeding/courtship displays are predicted to have a higher chance of being cuckolded (Reyer et al. 1997).

this is because in the conditions of the sub Arctic pairs will often pre-incubate incomplete clutches of 2 - 3 eggs during periods of adverse weather. Thus, members become separated and this may create a window of opportunity for unguarded females to copulate freely with extra-pair males as well as for the latter to seek EPCs. As a result of this early incubation, extra-pair young in broods will be more likely to come from eggs laid last. If, however, extra-pair fertilization is equally likely to occur at any time during a female's fertile period, then each egg in a clutch will have equal probability of being fertilized by a male outside of the social bond.

I tested these predictions by combining observations of mating behaviour of Semipalmated Plovers in the field with an examination of paternity using multilocus DNA fingerprinting in 24 plover families.

Study area. The study was conducted in June and July of 1996 and 1997 along a ca. 30 km coastal stretch of the Hudson Bay east of the town of Churchill, Manitoba (Canada) (58°45'N 95°04'W). The Semipalmated Plover breeding sites were on coastal mudflats, gravel ridges, lakeshore, and occasionally on tundra (Sullivan Blanken 1996) up to 8 km inland (Jehl & Smith 1970).

Field work. In this and other studies, behavioural observations alone cannot adequately assess the frequency of extra-pair paternity. These observations, however, can provide a background for understanding the mating behaviour and behavioural factors that may influence the frequency of EPP in the Semipalmated Plover. They should be interpreted in the context of the DNA fingerprinting data.

Field observations were conducted opportunistically with the help of 10x24 binoculars and a 20-40x spotting scope. Areas that appeared to be occupied by breeding pairs were searched no less than 2 - 3 times during the presumed incubation period starting after the injury feigning behaviour of adults was first observed. Nests were located by observing flushed adult birds when they returned to their nests to resume incubation. Positions of discovered nests were marked with natural objects such as rocks or dry

branches placed no closer than 10 - 15 m from a nest. As an attempt was made to find all existing nests in the study area, behavioural observations were made while searching for nests.

Distances between nest sites were measured with either a 30 m tape (under 100 m) or with a Garmin™ GPS receiver (over 100 m).

An intensive banding effort in the area was started in 1993 (Sullivan Blanken 1996) and in subsequent years ca. 50% of birds (Flynn 1997) returning to the area could be identified by a combination of one to three colour plastic bands and one Canadian Wildlife Service (CWS) aluminium band on their tarsi. In 1996 and 1997 adult birds were captured on nests with walk-in-traps and banded with an individual specific combination of three plastic colour bands and one aluminium CWS band. Chicks were caught upon hatching and banded with a brood specific combination of two plastic colour bands and one aluminium CWS band.

Dial calipers were used to take measurements (to the nearest 0.1 mm) of the bill (total length as well as the length of black and orange sections of the bill), tarsus length, width of the forehead band and length of the white supercillium. A ruler with a stop bar was used to measure wing length (to the nearest 0.5 mm) and a Pesola spring

scale was used to weigh individuals (to the nearest 0.2 g). The number of brown feathers in the breast and forehead bands was also counted (Teather & Nol 1997). All birds captured for the first time were assumed to be at least two years of age as no individuals banded as chicks, returned to the area to breed earlier than the age of two years (unpubl. data). Adult plovers were sexed primarily basing on the proportion of black feathers in their forehead and breast bands and the brightness of the proximal orange section of the bill. Males were always blacker and brighter than their mates (Cramp & Simmons 1983). They also had a much more clearly defined boundary between the black and orange sections of the bill.

Extra-pair as well as in-pair interactions between individuals were recorded only when at least one of the interacting birds could be identified by its band combination. Achievement of a cloacal contact between mating individuals was counted as a copulation. A pair was defined as an association between a male and a female tied to a particular area (territory) persisting for more than one day. All presumed pairs were confirmed by subsequent nesting of individuals under observation.

Pairs were divided into two sets of two categories and compared as either solitary versus neighbourhood nesting

pairs or single-purpose versus multi-purpose territory pairs, depending on breeding density and territory type respectively. Pairs nesting solely (i.e., no other birds nested within a 200-250 m radius) and utilizing individual feeding sites not shared with other conspecifics were termed solitary. I termed pairs nesting in loose aggregations which utilized a common foraging area as neighbourhood pairs (and the aggregations - neighbourhoods). If a breeding territory was used only as a nesting site and birds flew elsewhere to feed, that territory was considered single-purpose. Territories (usually coastal) used by pairs as both nesting and feeding sites were termed multi-purpose.

Breeding synchrony was determined as an average percentage of females that were fertile per day during the breeding season (Stutchbury & Morton 1995). The fertile period for a given female was conservatively defined as starting six days before laying of the first egg to the laying of the penultimate egg in the clutch (3 to 4 eggs in a clutch) (Stutchbury & Morton 1995).

Blood sample collection. I normally collected 100 to 150 μ l of blood from adult birds and chicks, although in some cases less blood (ca. 20 - 50 μ l) was obtained from chicks. From adults blood was drawn into a heparinized haematocrit microcapillary tube following a venipuncture of

the brachial vein (left wing). In the chicks blood was drawn into a syringe directly from the left jugular vein. Due to their very elastic skin this method turned out to be much easier to use (quicker) and potentially less damaging (lower chance of causing a haematoma) to the chicks than collecting blood using haematocrit microcapillaries. In both cases blood was expelled into a 1.8 ml Nalgene® cryogenic vial containing either 1 ml of the 'Queen's' lysis buffer (0.01 M Tris-HCl, 0.01 M NaCl, 0.01 M EDTA, 1% n-lauroylsarcosine, pH 8.0, Seutin et al. 1991) or 0.5 ml of the 'Oregon' blood-storage buffer (1 M Tris-HCl, 0.5 M EDTA, 5 M NaCl, pH 8.0) (T. Mullins, pers. comm.) and subsequently stored at room temperature until DNA extraction.

DNA extraction. DNA extraction and digestion were carried out as in Lifjeld et al. (1993) with minor modifications. Normally about ½ of the blood-buffer mixture was used for DNA extraction. The clot was placed in a 13 ml falcon tube and the total volume was brought up to 4 ml with the AB lysis buffer (0.1 M Tris, 4 M urea, 0.2 M NaCl, 0.01 M CDTA, 0.5% n-lauroylsarcosine, pH 8.0). The tube was left in a shaker overnight at 37°C ; the following day 500 µl of proteinase K solution (3.8 mg/ml) in a buffer (0.01 M Tris-HCl, 0.001 CaCl₂, pH 8.0) was added to the blood-buffer mixture and set in a shaker at 37°C for another 12 to 20

hours. After the lysis procedure a series of phenol-chloroform-isoamyl (25:24:1) extractions was performed. Four ml of phenol-chloroform-isoamyl were added to the tube containing 4 ml of lysed blood and shaken gently for 30 min. After mixing, the tube was centrifuged for 15 min at 5000 rpm. The supernatant was transferred into a new tube and the process was repeated. Subsequently the 4 ml of supernatant were purified with 100% chloroform following the same protocol as for phenol-chloroform-isoamyl extraction. DNA was precipitated by the addition of 1/10 volume (400 μ l) of 3 M NaAc solution (pH 5.3) and an equal volume (4.4 ml) of isopropanol. The mixture was inverted several times in the tube allowing the DNA to precipitate. The DNA pellet was transferred into a 1.5 ml microcentrifuge vial, washed with 70% ethanol and let dry for ca. 15 min or until semi-transparent. The dried DNA pellet was dissolved in 0.5 ml of 1x TNE₂ buffer (0.01 M Tris-HCl, 0.01 M NaCl, 0.002 M EDTA, pH 8.0) in a water bath at 37°C for 24 to 48 hours. Roughly 30% of all DNA samples were extracted using a QIAamp Tissue Kit (QIAGEN) following the manufacturer's instructions. After the complete resuspension of the pellet the initial estimate of DNA concentration and purity was made using a Gene Quant II (Pharmacia Biotech)

spectrophotometer. Only samples with A_{260}/A_{280} absorption ratio of 1.6 or higher were used for analysis.

DNA integrity and concentration were estimated by running a selection of samples (uncut and *EcoRI* digested respectively) against DNA of known concentration for ca. 1 hour on 7x10 cm 0.8% 10 µg/ml ethidium bromide stained agarose gels in 0.5x or 1x TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0).

DNA digestion. An aliquot containing ca. 15 - 20 µg of DNA was brought to the final volume of 300 µl by adding ddH₂O, 30 µl of the enzyme buffer (1/10 of the final volume) and 3-4 µl of the restriction enzyme (Hae III, 10 U/µl, SIGMA). Digestion was allowed to proceed for 6 hours to overnight in a water bath at 37°C with flicking and spinning every hour for the first 2 - 3 hours. The following morning 1/10 volume (30 µl) of 3 M NaAc (pH 5.3) solution and 2.5 volumes (750 µl) of 95% ethanol were added to the digestion mixture. Contents of the microcentrifuge vial were gently mixed and placed in a freezer at -20°C for a minimum of 5 hours. The vials were centrifuged for 30 min at 13200 rpm. Ethanol was poured off and the remaining DNA pellet was left to dry for 30 min to 1 hour and then resuspended in 30 µl of 1x TNE₂ buffer for at least 1 hour at 37°C with intermittent flicking and spinning. Following resuspension of digested

DNA, 3 μ l of the DNA solution were run on a 7x10 cm 0.8% agarose gel (10 μ g/ml EthBr stained) to check for complete digestion of the samples and balance out the concentration among samples if needed. Comparisons to previously run gels with control samples of known concentration were also made. Resuspended digested DNA samples were either immediately used or stored at -20°C.

Gels. Gel running, Southern blotting, and hybridization procedures were conducted following the protocol described by Gurlich et al. (1991) with minor modifications. A 20 x 35 cm custom made plexiglass gel box was used for running gels. Digested DNA was separated in 0.8% agarose 1xTBE buffer gel at 65 - 70 V over the 36 - 40 hour running time or until the bromophenol blue (0.25% bromophenol blue, 40% (w/v) sucrose in water) front migrated beyond the 25 cm line. Each lane was loaded with ca. 5-7 μ g of predigested DNA with addition of 10 ng of lambda *Hind*III, *Hind*III + *Eco*RI, *Bst*EII digests used as in-lane markers. Adenovirus *Hind*III digest (200 ng) loaded in outside lanes was used as the external size marker. Prior to running, all samples were pre-heated at 65°C for 5 min to avoid the occurrence of "sticky ends", while the gel itself was pre-run at the working voltage for ca. 1 hour.

Southern Blotting. Upon completion of a run a DNA fingerprint gel was trimmed with a scalpel or razor blade removing the wells and empty lanes as well as the portion of the gel above the 25 cm line. Acid nicking. The gel was placed in a tupperware dish filled with 500 ml of 0.25 N HCl and left there for 15 minutes at room temperature with gentle agitation. Denaturing. The acid solution was removed and the gel briefly rinsed with distilled water. Then 500 ml of the denaturing solution (0.5 M NaOH, 1.5 M NaCl) were added to the dish and left shaking gently at room temperature for 1 hour. Neutralization. After the denaturing solution was poured off the gel was briefly rinsed with distilled water and 500 ml of the neutralizing solution (1 M Tris-HCl pH 8.0, 1.5 NaCl) were poured into the dish and left to shake gently for 1 hour at room temperature.

The upward transfer Southern blotting apparatus was constructed during the neutralizing step. A piece of nylon transfer membrane (Immobilon-N) was cut to the size of the gel. The membrane was wetted in 95% ethanol for 5 sec, rinsed with distilled water until hydrophilic, and then soaked in 10x SSC (1.5 M NaCl, 0.15 M sodium citrate) for 10 min. The upward DNA transfer using 10x SSC as a working solution was performed from 6 hours to overnight. Briefly,

the gel was placed on 6 20x47 cm filter paper (Whatman No. 1) wicks equilibrated in 10x SSC for 10 min, overlaid with the transfer membrane, 6 filter paper squares cut to the size of the gel and a stack of paper towels. A 0.5 kg weight was placed on top of paper towels. The paper towels were replaced 1 and 4 - 5 hours after the start of the transfer procedure. The following morning the blot was rinsed in 10x SSC for 5 minutes, air dried for 30 min (room temperature) and baked at 80°C for 1 hour.

After baking, blots were sealed in plastic until ready to be used.

Hybridization of a $\alpha^{32}\text{P}$ -labelled multilocus probe (Jeffreys' 33.15) to a DNA blot. Jeffreys' 33.15 (Jeffreys et al. 1985a, b) insert in M13 phage plasmid was used as the multilocus probe. Jeffreys' 33.15 multilocus probe is a 17-base pair long core sequence repeated 29 times without flanking regions that was cloned out of the human myoglobin locus.

Pre-hybridization was carried out at 64.5°C for 4 hours to overnight in 10 - 12 ml of the Westneat's hybridization solution (7% SDS, 0.001M EDTA, 1% BSA (fraction V), 0.25M Na_2HPO_4) (Westneat et al. 1988). Radioactive labelling procedures were performed in accordance with the manufacturer's instructions (Pharmacia Biotech). Briefly,

25 ng of dissolved plasmid DNA were denatured and subjected to the random primed labelling (Feinberg & Vogelstein 1983) in the presence of the oligonucleotide Reagent Mix (10 μ l), Klenow fragment solution (1 μ l, 5-10 units/ μ l), [α - 32 P] dCTP (5 μ l, 3000 Ci/mmol) (Mandel Scientific Inc.) and ddH₂O in the total reaction volume of 50 μ l. Labelling was allowed to proceed for 1 hour at 37°C. Unincorporated label was removed by passing the solution through a G-50 Sephadex column. Specific activities were typically > 1.0 x 10⁹ cpm/ μ g. Hybridization was carried out at 64.5°C for 20 hours. Following hybridization the blots were washed in 2x SSC, 0.1% SDS: once at room temperature for 15 min and twice at 64.5°C for 15 min, then for 30 min. After rinsing in 2x SSC blots were exposed to Boehringer Mannheim chemiluminescent X-ray film first for 24 hours to assess the required full exposure time, then for 3, 7 and/or 14 days at -80°C.

Autoradiographs were developed using the GBX developer and fixer (Kodak Inc.) following the standard manufacturer's instructions, i.e., 5 min developing, 1 min stop-bath (3% acetic acid solution), 5 min fixing, and 15 min washing in running water.

Fingerprint scoring procedures and analysis.

Fingerprint scoring was not done blindly, i.e. the scorer

knew which lanes contained offspring and which putative parents (Westneat 1993a). DNA samples of chicks were always run on the same gel and flanked by those of their putative parents. Thus, the distance between samples of a parent and any of its presumed chicks was no more than 3 lanes (a full Semipalmated Plover family consists of 2 parents and 4 chicks). When analysing DNA profiles, all bands in the 2.0 kb to 20.0 kb region were identified. Two bands were considered to match if they displayed no more than a two-fold difference in intensity and their centres differed in electrophoretic mobility by less than 0.5 mm (Bruford et al. 1992, Westneat 1990). All available pair male-female dyads were presumed to be unrelated and thus were used to calculate the background band sharing coefficient.

Scoring was performed by placing acetate overlay sheets over the developed autoradiographs and marking bands with coloured pens. Different colours for paternal, maternal, mixed, and novel bands were used. In all cases scoring was conservative, that is if possible bands of chicks were matched with bands in their putative parents. Hatchling bands not present in a social parent had to be clearly different to be considered novel fragments (Westneat 1993a).

Parentage and relatedness were analysed through band sharing coefficients (D) and when both putative parents were

available, through the number of novel bands, that is offspring bands unmatched by those detected in social parents (Westneat 1990). Band sharing was determined using the equation $D = 2N_{ab}/(N_a + N_b)$ (Wetton *et al.* 1987, Bruford *et al.* 1992) where N_a and N_b are total numbers of bands for individuals a and b respectively and N_{ab} is the number of bands with similar intensity and electrophoretic mobility shared by the individuals a and b . Three or more novel bands in an offspring coupled with low band sharing (i.e., close to the background band sharing coefficient) between the offspring and a parent (father) were deemed sufficient to consider it extra-pair.

A full linkage analysis was not carried out because a large family of known parentage was not available (Amos *et al.* 1992). Thus the true extent of co-segregation among fragments of different lengths was not known. However, the number of discerned bands was high, separation between mean band sharing values of unrelated individuals and first-order relatives was clear, and the distributions of band sharing coefficients for father-offspring and mother-offspring pairs did not differ significantly signalling that there was no predominant sex linkage. This means that the risk of an erroneous assignment of parentage to an unrelated individual

due to inflated band sharing resulting from fragment linkage was negligible.

The expected band sharing value among first order relatives in the population was calculated using the following formula: $S = \theta + r(\theta - 1)$ (Lynch 1991) where θ is the background band sharing coefficient and r is the proportion of bands identical by descent between two individuals ($r = 0.5$ for a parent-offspring pair).

Normally distributed data were analysed using parametric tests, otherwise non-parametric tests were employed. MINITAB™ and STATISTICA™ software packages were used.

RESULTS

Pair formation and mating behaviour in the Semipalmated Plover. In 1996 the first Semipalmated Plovers were recorded in the study area on 28 May. In 1997 the field season was started later (2 June) when some birds were already present in the area. Pair formation in Semipalmated Plovers at the study area in Churchill commenced immediately upon arrival of both potential partners to a breeding territory/site. In most cases specific breeding sites were first occupied by males - one to three days before the appearance of females. However, there was no significant difference in arrival dates of male and females to the general area: males arrived only 0.3 day earlier than females - both Julian day 156 (June 5) (Mann-Whitney test, $P > 0.05$, males: June 5.25, $SD=2.7$, female: June 5.54, $SD=2.7$). The overall sex ratio in the study area was close to 1:1. In both years, however, there was a small proportion of males (3 (7.5%) in 1996; 4 (6.0 %) in 1997) that did not manage to secure territory and/or mates. One of the lone males in 1997 maintained a breeding territory where demonstration scrapes were built, but he failed to attract a mate.

The approximate breeding pair density was 0.18 pair/km² in 1996 and 0.23 pair/km² in 1997 for the total area of 384

km² (Flynn 1997). The distribution of birds, however, was not random with most of the breeding pairs clustered in a few pockets of suitable breeding habitat (dry tundra, eskers, gravel patches) along the coast and near inland lakes.

In both years most pairs nested in neighbourhoods rather than solitarily (Table 1). Both multi-purpose and single-purpose territories were established by males in almost equal proportions (Table 1); in the latter case two sites: (1) feeding and (2) nesting, separated by a swath of unsuitable habitat were maintained. During the pair formation period, foraging sites of pairs occupying single-purpose nesting territories were used for courting. Males constructed two or more 'demonstration' scrapes to which they attempted to attract females by engaging in frequent display flights over the courtship sites. The flights were accompanied with prolonged calling. Associations between males and females were formed very quickly - within a day of the first appearance of a female at a (foraging) site defended by a male. Females began by feeding within their prospective mates' territories. They eventually indicated acceptance of the male (territory) by lowering themselves in the demonstration scrapes and mimicking nest-building behaviour: substrate scraping, nesting-material tossing and

Table 1. Numbers and proportions of Semipalmated Plover pairs (solitary and neighbourhood and occupied territories (single-purpose and multi-purpose) observed near Churchill, Manitoba, Canada in 1996-97.

YEAR	Pair Type		Territory Type		Total
	Solitary	Neighbourhood	Single-purpose	Multi-purpose	
1996	9 (22.5%)	31 (77.5%)	19 (47.5%)	21 (52.5%)	40 (100%)
1997	12 (22.6%)	41 (77.4%)	28 (52.8%)	25 (47.2%)	53 (100%)
TOTAL	21 (22.6%)	72 (77.4%)	47 (50.5%)	46 (49.5%)	93 (100%)

vocalizations. Demonstration scrapes appeared to be simpler and more elaborately lined with nesting material than true nesting depressions. Copulations usually took place in the vicinity of demonstration scrapes following a courtship flight. The flight, similar to male-alone courtship flights (see Sullivan Blanken 1996 for a description) lasted one to five minutes and birds usually copulated immediately upon landing. The general copulation routine was similar to that observed in other plover species, e.g. the Ringed Plover *Charadrius hiaticula* (Cramp & Simmons 1983) or the Piping Plover *Charadrius melodus* (Cairns 1982, per. obs.). It started with "high-stepping" by the male, followed by the assumption of the crouched pre-copulatory position by the female, then mounting and, finally, the cloacal contact. A total of 13 in-pair and one extra-pair copulations were noted over 30 hours of observation during the presumed females' fertile period in two years (1996 - 1997). IPCs occurred at a frequency of 0.43 copulations per hour of observation. In one case a pair performed 2 copulations in 1 hour. The average duration of an in-pair copulation was 48.6 sec (n=9, SD=14.3, range=29-73). Time elapsed between observed copulation and clutch initiation in the pairs was on average 8.9 days (n=7, SD=7.1, range=1-18). Overall breeding synchrony was 38.3% in 1996 (n=29 females) and

46.7% in 1997 (n=36 females) reaching maximum day values of 70 and 94% respectively during the second week of June in both years.

Nesting territories or nesting sections of multi-purpose territories were used in a more secretive manner, i.e., usually no courtship displays were performed in the vicinity of nests. The distance between the courtship/feeding and nesting territories occupied by the same pair ranged from 0 (when a multi-purpose site was used) to ca. 600 m. The distance between courtship and nesting scrapes was determined for seven pairs (single-purpose and multi-purpose territory holders combined) and on average equalled 221.4 m (n=7, SD=185, range=70-610).

No mate switching was observed. Once two birds formed an 'association' it was maintained for the duration of at least one breeding season. If a clutch was lost and a renest was initiated pair-bonds were maintained (Flynn 1997, pers. obs.).

Mate-guarding appeared to be very intensive during the pre-laying period with males following females and staying close to their mates although this behaviour was not quantified. Females flying from one site to another were always followed by their mates. Only brief separations between paired birds were ever observed (usually when the

male engaged in a territorial display with or a chase after another male). Territorial intrusions by both sexes seemed frequent and normally resulted in a quick expulsion of intruding birds.

The only observed extra-pair copulation (7.1%) occurred on 9 June 1997, early in the laying period. A paired female feeding on the mudflats was briefly courted by an extra-pair male who subsequently mounted her and achieved a cloacal contact. No apparent solicitation by the female was observed and the extra-pair male appeared to be unpaired at that moment. Immediately after the two birds separated the social mate of the female arrived and drove the intruder away. He then courted the female and copulated with her. The copulation took place in a courtship/feeding area. Later in the season the pair moved out of the courtship/feeding area and was only relocated after their brood reached the age of ca. 14 days so that neither of the adults could be trapped.

In 1997 an unpaired female attempted to solicit an EPC from a paired colour-banded male who appeared to be a winner in a territorial encounter with another bird. The female assumed a crouching position (a typical female plover pre-copulatory position) in front of him but was attacked by the paired colour-banded male after his own mate appeared at the

scene. Later this unpaired female copulated with an unpaired male who seemed to be the only mate available in the area, thus presumably forming a pair. An interesting observation was made in 1997 when a paired colour-banded female who had already laid three out of four eggs in her clutch arrived to a feeding area while her mate was incubating the clutch. Soon upon landing this female directed a 'high-stepping' display (a typical male plover pre-copulatory behaviour) at an extra-pair male and attempted to mount him. This behaviour (possibly solicitation) elicited a response in the male. He initiated a pre-copulatory display directed at the female and she appeared to be ready to accept the copulation as a pre-copulatory posture was assumed. No copulation, however, was achieved as two other birds (a male and a female) entered the scene invoking territorial displays between the males.

A territorial male who did not manage to secure a mate continued his territorial and courtship displays/flights throughout the breeding season and attempted to copulate whenever a female (in all three occasions an already nesting paired off-duty bird) landed within his territory to feed. In all cases the females were not cooperative and were subsequently attacked and driven away by the unpaired male. At least 15 observations were made when either an in-pair or

extra-pair copulation attempt failed due to lack of cooperation on behalf of the female and each time the latter was attacked and/or hit/pecked by the male.

A case of polyandry was suspected (but not confirmed) when two males were routinely observed near one of the nests. One of them and the female were subsequently captured and colour-banded. Depredation of the nest early in the season, however, prevented me from capturing/banding the second male or verifying his role at the nest via observation of incubating birds.

Out of six unhatched eggs examined in 1997 (7% of 85 eggs that survived through hatching), five contained dead embryos in early stages of development; the cause of failure of the remaining egg could not be established based on visual examination alone. Thus, at maximum only one unfertilized egg could be found.

When comparing morphometry of solitarily versus neighbourhood-nesting and single-purpose versus multi-purpose territory males (Tables 2, 3) no significant difference between any of the analysed parameters was found. Females using single-purpose territories had significantly longer wings than the birds from multi-purpose territories (Table 2). However, two-way MANOVA analysis of morphological traits of males and females by nesting type

Table 2. Comparison of six morphological traits and age for the Semipalmated Plovers occupying single-purpose and multi-purpose nesting territories near Churchill, Manitoba, Canada (1996-1997).

	Male			Female		
	Single-purpose x (SE, n)	Multi-purpose x (SE, n)	P ¹	Single-purpose x (SE, n)	Multi-purpose x (SE, n)	P ¹
Weight (g)	45.9 (0.42, 29)	46.5 (0.39, 30)	ns	48.1 (0.34, 26)	47.4 (0.50, 32)	ns
Tarsus (mm)	24.5 (0.15, 29)	24.7 (0.13, 30)	ns	24.5 (0.16, 27)	24.0 (0.16, 32)	ns
Wing (mm)	123.3 (0.48, 29)	123.3 (0.54, 30)	ns	126.2 (0.50, 27)	124.4 (0.50, 32)	0.016
Bill (mm)	12.1 (0.22, 29)	12.1 (0.24, 30)	ns	12.1 (0.09, 27)	12.0 (0.09, 32)	ns
Condition (g/mm)	1.86 (0.02, 29)	1.88 (0.02, 30)	ns	1.96 (0.01, 26)	1.97 (0.02, 32)	ns
Wing load (g/mm)	0.37 (0.00, 29)	0.38 (0.00, 30)	ns	0.38 (0.00, 26)	0.38 (0.00, 32)	ns
Age ²	3.14 (0.30, 29)	3.07 (0.22, 30)	ns	2.93 (0.26, 27)	2.66 (0.23, 32)	ns

1. Two-sample t-test with sequential Bonferroni correction, CI=95.0%.

2. Assuming the age of first breeding as 2 calendar years.

Table 3. Comparison of six morphological traits and age for the solitarily and neighbourhood breeding Semipalmated Plover pairs near Churchill, Manitoba, Canada (1996-1997).

	Male		P^1	Female		P^1
	Solitary, x (SE, n)	Neighbourhood, x (SE, n)		Solitary, x (SE, n)	Neighbourhood, x (SE, n)	
Weight (g)	45.9 (0.71, 14)	46.4 (0.31, 44)	ns	47.9 (0.57, 15)	47.7 (0.39, 43)	ns
Tarsus (mm)	24.3 (0.22, 14)	24.7 (0.11, 44)	ns	24.4 (0.26, 15)	24.2 (0.13, 43)	ns
Wing (mm)	124.6 (0.90, 14)	122.9 (0.37, 44)	ns	126.3 (0.54, 15)	124.8 (0.45, 43)	ns
Bill (mm)	12.0 (0.45, 14)	12.3 (0.07, 44)	ns	12.1 (0.14, 15)	12.1 (0.10, 43)	ns
Condition (g/mm)	1.89 (0.03, 14)	1.88 (0.01, 44)	ns	1.96 (0.20, 15)	1.98 (0.20, 43)	ns
Wing load (g/mm)	0.37 (0.00, 14)	0.38 (0.00, 44)	ns	0.38 (0.00, 15)	0.38 (0.00, 43)	ns
Age ²	3.43 (0.49, 14)	3.02 (0.20, 44)	ns	2.87 (0.34, 15)	2.74 (0.20, 43)	ns

1. Two-sample t-test with sequential Bonferroni correction, CI=95.0%

2. Assuming the age of first breeding as 2 calendar years.

(solitary vs neighborhood) and territory type (single- vs multi-purpose) detected no significant differences in any of them (Wilk's Lambda=97, df=5, $p=0.65$ and Wilk's Lambda=0.95, df=5, $p=0.31$ respectively).

Parentage analysis. DNA fingerprints from a total of 24 Semipalmated Plover families, 7 from 1996 and 17 from 1997, comprising a total of 130 individuals (45 adults and 85 chicks) were obtained. The average number of scorable bands observed in an individual was 27.3 (SD=4.8, range 15 - 39). The band sharing values (Table 4) did not differ significantly between male-offspring and female-offspring pairs (t-test, $p=0.62$, df=136). Single novel bands were observed in two chicks (from different families) out of 62 matched by both parents, resulting in the probability of observing one chick with a novel band of 0.032. The probabilities of observing offspring with two and three novel bands were 0.001 and 0.00003 respectively. This means that, with our sample size the expected number of offspring with these numbers of bands resulting from random processes (mutation, scoring error) alone will be 0.06 and 0.002 respectively. Indeed no chicks with 2 or 3 novel bands were found. There was a clear separation between ranges of band sharing coefficients of unrelated adults and parents and

Table 4. Band sharing coefficients (D) between pairs of Semipalmated Plovers whose relationship has been determined by band exclusion.

	D	S.D.	95% CI	N
Unrelated adults	0.18	0.05	0.16-0.20	20
Parent-Offspring ¹	0.60	0.08	0.58-0.61	147
Male-Offspring	0.60	0.08	0.58-0.61	81
Female-Offspring	0.61	0.08	0.58-0.62	66
Father-EPY	0.24	0.03	0.18-0.29	4
Mother-EPY	0.64	0.09	0.52-0.77	4
EPY-EPY	0.65	0.08	0.44-0.85	3

1. The expected D value for first-order relatives (parent-offspring in our case) was determined to be 0.59 after Lynch (1991).

their offspring (Fig. 1). For the former the range was between 0.05 and 0.28 while for the latter: 0.42 and 0.77. This allowed me to establish two criteria for parentage exclusion: (1) three or more novel bands and (2) the conservatively chosen band sharing coefficient with the putative parent of 0.35, roughly the mid-point between maximum and minimum values of the background and parent-offspring band sharing coefficients respectively.

Often novel bands are a result of random mutations (Burke & Bruford 1987). Presuming that the unique bands found in two hatchlings with determined parentage resulted from mutations the mutation rate in the species is 0.0012 per meiotic event (at least for the loci concerned) which is at the lower end of the rates reported elsewhere (e.g. Burke & Bruford 1987).

Out of 85 hatchlings examined 66 were from full families while for the remaining 19 only DNA of putative fathers was available. Since no instances of ISBP were suspected in the population and subsequently discovered in 66 young (Fig. 2), only the father-offspring band sharing coefficient was used for the 19 chicks to determine their paternity.

A total of four chicks (4.7%, 4/85), all coming from the same brood (4.1%, 1/24) satisfied my conditions for

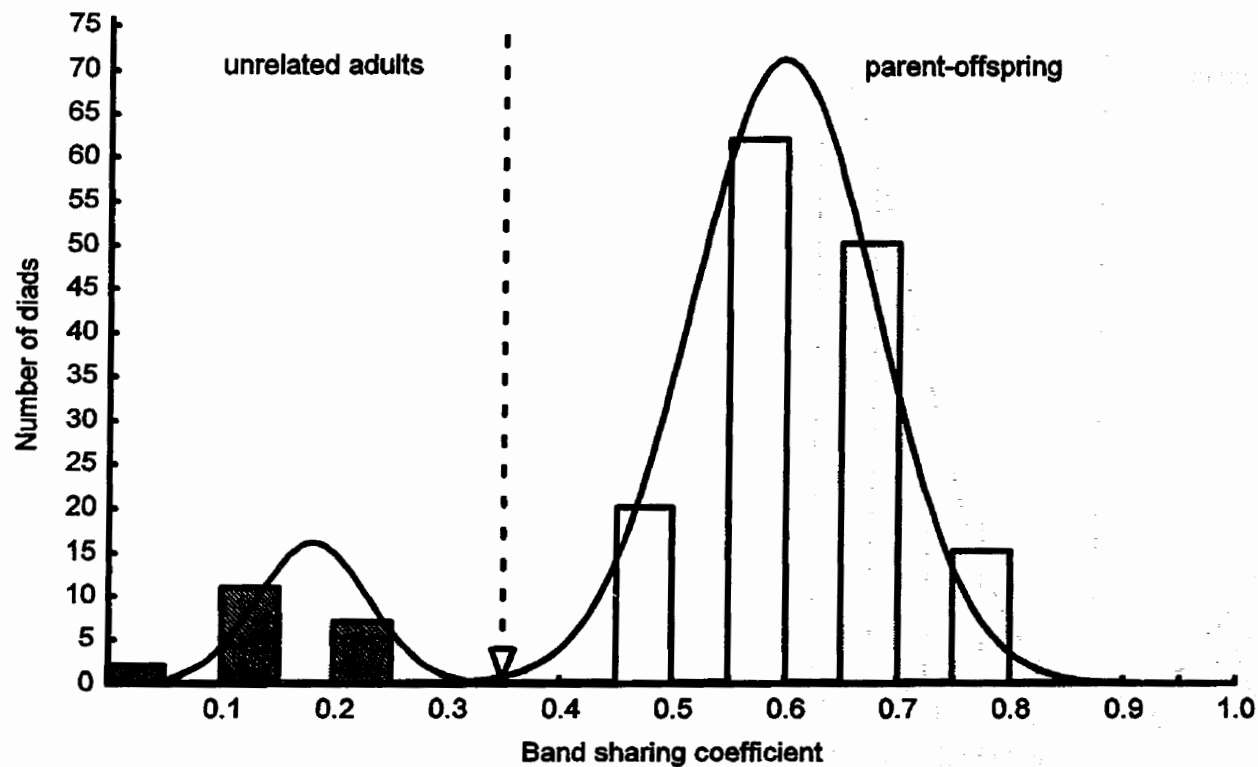


Figure 1. Band sharing coefficient frequency distribution for unrelated adult (n=20) and parent offspring (n=147) dyads. The dashed line indicates the separation point between the ranges of the two sets of values. The solid line represents a fitted normal distribution.

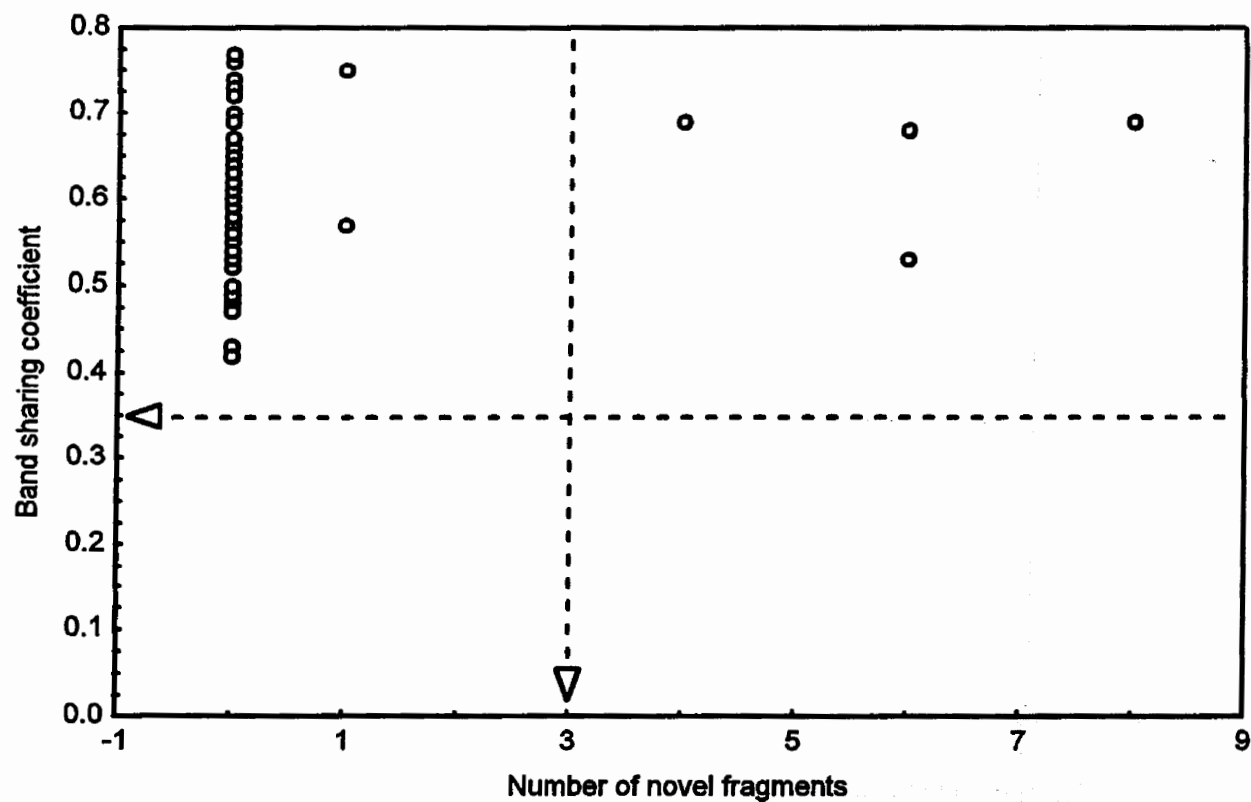


Figure 2. The relationship between the social mother-offspring band sharing coefficient and the number of novel bands in the offspring (n=66). The dashed lines indicate the criteria for parentage exclusion. The four data points in the upper right corner represent extra-paternal young.

extra-pair paternity assignment (Fig. 3, 4). They possessed between 4 and 8 novel bands and their father-offspring band sharing coefficient ranged from 0.19 to 0.27 while the mother-offspring coefficient was well above 0.5 (Table 4).

The pair with extra-pair paternity bred in 1996 in a neighbourhood and used a multi-purpose territory. Both members of the pair were experienced breeders. Both birds were significantly older than the rest of the population (male 5 years versus 3.0 ± 1.39 , female 4 years versus 2.65 ± 1.17 respectively, one sample t-test, $P < 0.05$). Their individual morphological characteristics, however, did not differ significantly from the population means (one sample t-test, $P > 0.05$). In 1997 the female member of the pair was not seen in the area while the male returned to the same nesting site and repaired with a different female that was also present in the area in 1996. The 1997 brood had a completely in-pair parentage.

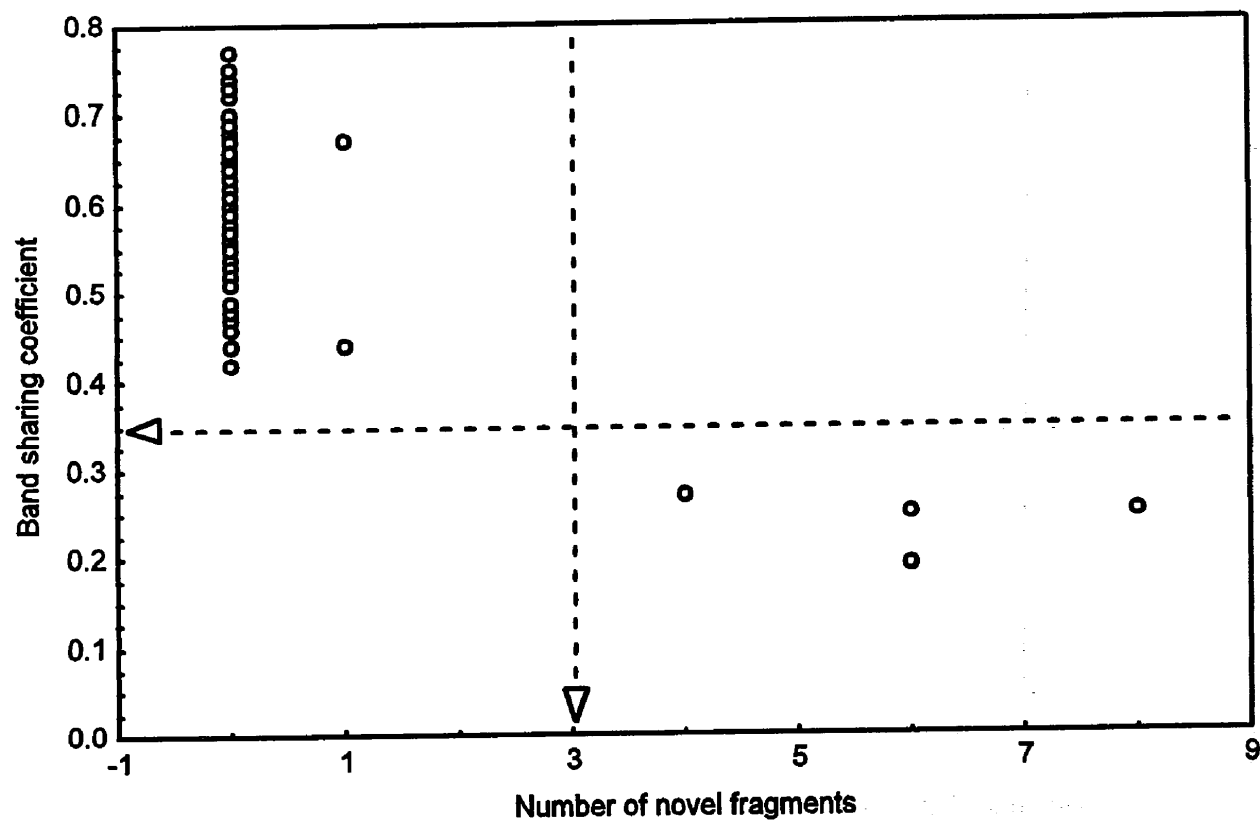
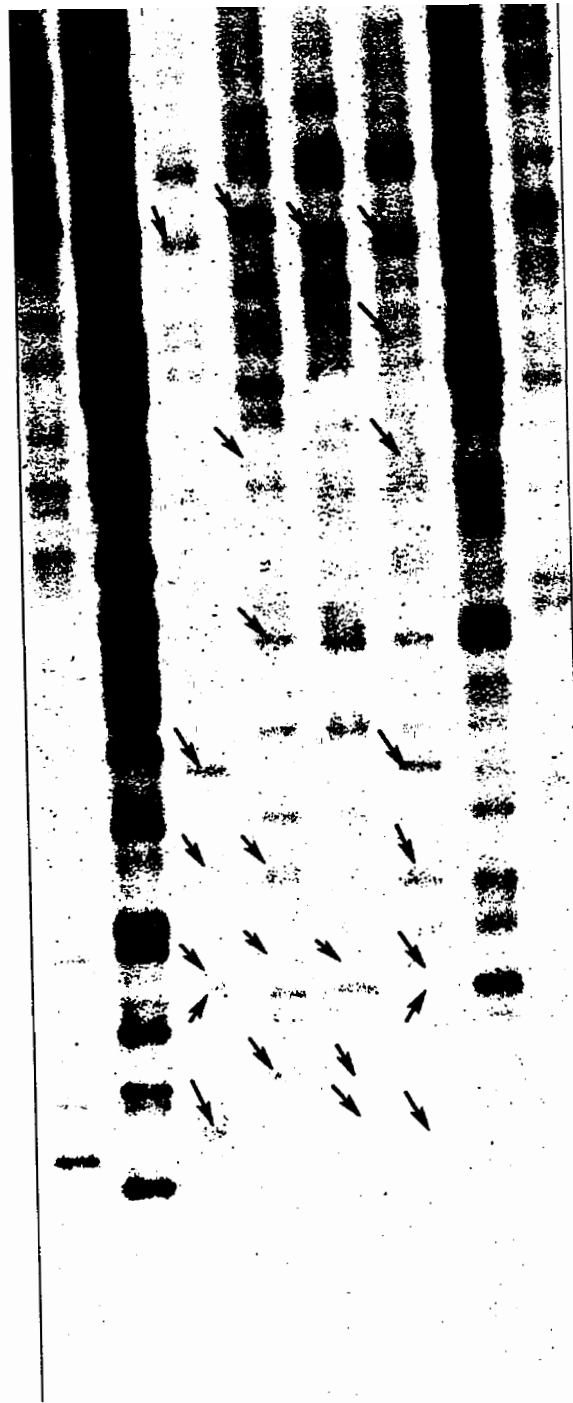


Figure 3. The relationship between the social father-offspring band sharing coefficients and the number of novel bands in the offspring (n=66). The dashed lines indicate the criteria for parentage exclusion. The four data points in the lower right corner represent extra-paternal young.

Figure 4. An example of a fingerprint (Jeffreys 33.15 multilocus probe/*HaeIII*) of a Semipalmated Plover family (M - social father, 01-04 - offspring, F - social mother) with extra-pair young. Arrows indicate novel bands that with the absence of egg-dumping in the population were presumed to be extra-pair paternal.



2.0kb

20kb

DISCUSSION

No single explanation can be given as to why some plovers prefer to nest solitarily (usually inland) and use single-purpose territories while others nest in loose aggregations (usually coast) and utilized multi-purpose territories. Nor is there much quantitative information that could support a specific view. Below, however, I provide some possible explanations for the observed differences in nesting.

There appears to be an abundance of breeding sites in the area which would either force individuals to nest more densely or lead to creation of a 'sink' population nesting in suboptimal conditions. Neither area seems to offer better protection from predators as neither inland nor coastal sites can be characterized by a consistently higher hatching/fledging success (E. Nol, unpubl. data, pers. obs.). Some differences, however, do exist. Coastal sites have higher food availability while the inland area is slightly warmer (1°C) allowing for earlier nesting (Sullivan Blanken & Nol 1998).

Apparent differences in female morphological traits (wing and tarsus lengths) were found between different types of territories but they were not very pronounced as only one of the univariate statistical tests used detected some (wing

length differences. Why is it then, that two different modes of nesting exist?

Both modes of nesting confer certain costs and benefits. Possible costs to single-purpose territory users of both sexes include higher energy expenditure due to the extended travel between nesting and feeding sites. For males using single-purpose territories there may be a greater possibility of cuckoldry due to more frequent opportunities for their mates to be left unguarded, especially at the end of egg-laying period. When, however, one considers possible benefits resulting from solitary nesting, it becomes apparent that the cost of extra travel may be offset by a much lower rate of territorial encounters for both sexes, but especially so for males who invest disproportionately more energy into territorial fights than do females (Sullivan Blanken & Nol 1998, pers. obs).

It is probably also more advantageous for birds to feed on the coast as the food availability is greater at coastal mudflats (Sullivan Blanken & Nol 1998). Females using multi-purpose territories also have longer wings which may further balance out the energy spent for inter-territorial flights. Flight is energetically less costly in longer winged individuals (Castro & Myers 1988). It may be that for yet unknown reasons some males may prefer to undertake

longer foraging flights over engaging in extensive territory defense interactions and thus select isolated/single-purpose nesting sites to minimize energy expenditure due to male-male competition. These sites may be accepted by longer-winged females because the quality of these males is not different from the ones nesting on the coast. The possible cost to males in terms of paternity is neutralized by their intensive mate-guarding during the pre-laying period.

The discovery of extra-pair paternity in 4.1 % (1/24) of Semipalmated Plover families (an EPP rate of 4.7%, 4/85) seems to support my prediction of a low to absent proportion of extra-pair young in Semipalmated Plover families in the study area near Churchill if compared to other species (Birkhead & Møller 1992). Extra-pair paternity may result from EPCs, rapid mate switching and, in some cases, from sequential or parallel polyandry. I believe that EPP in the family in question resulted from an EPC as (1) no mate switching was observed during either this or previous studies (Flynn 1997) and (2) although polyandry was suspected in one breeding effort during the study period, no other males were observed in a stable association with the pair in question. Whenever some relationship was found between cuckoldry rates and age of individuals, those were younger males who lost paternity significantly more

frequently (e.g. Wagner et al. 1996). In this study the cuckolded male was older than an average male in the population (5.0 versus 3.0 years). However, with only a single cuckolded male it is difficult to suggest whether in the Semipalmated Plover paternity loss is related to age or not.

The fact that all four chicks of the brood were sired by one extra-pair male, similar to that observed in Indigo Bunting (*Passerina cyanea*, Westneat 1990) and Yellow Warbler (*Dendroica petechia*, Yezerinac et al. 1995) may indicate that (1) chances of the last eggs in a clutch to be fertilized by an extra-pair male are probably no different from the first ones, (2) as extra-pair copulations were much less frequent than IPCs, a single copulation (EPC in this case) seems capable of fertilizing an entire clutch (Birkhead et al. 1987), and (3) some extra-pair copulations may be disproportionately more successful than IPCs (Birkhead et al. 1988).

The observation of an extra-pair fertilization in a neighbourhood and not among solitary nesting pairs agrees with the generally postulated positive correlation between breeding density and EPP rates in birds (Møller & Birkhead 1993a). This is because neighbourhoods/coastal sites boast

higher numbers of territorial birds per unit area than do inland areas especially during the pre-laying period.

Egg-dumping was never detected as examined clutches never exceeded the modal size of 4 eggs. This was fully supported by results obtained via DNA fingerprinting - all chicks could be matched to their putative mothers.

The low EPP frequency found in the Semipalmated Plovers may represent the outcome of a strategy where individuals pursuing EPCs are continually scrutinized by those striving to counter them (Heg et al. 1993). Several other, not necessarily mutually exclusive factors may also be involved in the generally low EPP rate in the Semipalmated Plover.

Paternity guards. Mate-guarding is a widely studied and recognized mechanism of paternity assurance in monogamous and polygynous birds (Westneat et al. 1990). The possible functions of mate guarding include prevention of other males' access to females and an obstacle for the latter to seek EPCs and/or assess the quality of prospective extra-pair partners (Slagsvold & Lifjeld 1997). A negative correlation between mate-guarding or the male presence near the female and the EPC rate has been observed in many species (e.g. Heg et al. 1993, Freeland et al. 1995). My observations seem to indicate that male Semipalmated Plovers in most cases effectively use mate-guarding to protect their

paternity, the best evidence of which is the generally low EPP rate.

Mate-guarding may serve a dual purpose. First it prevents an extra-pair male accessing to a paired female and second it inhibits females from seeking EPCs and/or assess extra-pair mate quality. This situation is known as a sexual conflict (e.g. Krokene et al. 1996). It is not clear to what extent sexual conflict is present in Semipalmated Plovers but an observation of a female possibly initiating an EPC while her mate was incubating, as well as loss of paternity in one of the families indicated that it does exist. Notwithstanding this, the overall DNA fingerprinting data showed that male Semipalmated Plovers are able to maintain sole access to their females (Burke et al. 1989, Davies 1992) and/or efficiently correct for overlooked extra-pair interactions with retaliation copulations (Møller & Briskie 1995).

Mate-guarding or presence of males within close visual range of their mates may protect the latter from being harassed by potential EPC seekers. This protection may be advantageous to females as it prevents them from stress during the pre-laying - laying period when their energetic demands are especially high. In this case females should follow males as frequently as they are followed themselves.

Male antagonistic and guarding displays may provide females with an additional means of assessing general male quality. In the Semipalmated Plover this may be especially true during the courtship period when several males in a neighbourhood may display simultaneously.

Often associated with mate-guarding in this species was physical reprisal. On at least 10 occasions males were noticed to hit and strike either uncooperative in-pair females or females previously approached by an extra-pair male. A similar behaviour was observed in five other cases when a female rejected extra-pair courting. A similarly interpreted behaviour by males coupled with subsequent copulations was observed in Zebra Finches *Taeniopygia guttata* following extra-pair contact (Birkhead et al. 1988). In-pair and extra-pair males may 'punish' uncooperative females for different reasons though. For an in-pair male physical punishment may be a way of discouraging contact of a female with other males, facilitating in-pair copulations or may even be an element of courtship. Extra-pair males may also employ reprisal to make it more costly for females to reject a copulation as well as expel an uncooperative female from a defended territory, thus vacating space for other females. If physical punishment is an effective tool in coercing copulations, then both male-initiated IPCs and

EPCs should be equally frequent, which was not the case. The existence of mate-guarding and the apparently generally low time/energy investment in extra-pair interactions by both males and females in the species, however, may be the factors that dramatically skew this ratio in favour of IPCs.

The other general paternity assurance method in birds includes frequent in-pair and retaliation copulations (Møller & Birkhead 1993a, Møller & Briskie 1995). In a few situations when either a foreign male approached and attempted to court a paired female or the latter became separated from her mate the in-pair male almost invariably initiated a courting display and often attempted to copulate with the female (n=5). This behaviour has been observed in a number of species (e.g. Birkhead *et al.* 1987, Møller 1987, Nol & Humphrey 1994). It makes sound biological sense as there is abundant evidence that the last copulating male typically fertilizes eggs by displacing or overlaying the previously deposited sperm (Birkhead *et al.* 1988, Hunter *et al.* 1992 and references therein). In the Semipalmated Plover, where courting often takes place in common courtship/feeding areas where several potential pairs are present, some males cannot rely on mate-guarding alone as territorial encounters are frequent and paired individuals sometimes become separated. Also, as discussed above,

certain females may actively seek EPCs, especially with males that appear to be better competitors during territorial fights and thus retaliation against sperm from an intruder should provide a good complementary tactic to mate-guarding. The fact that in-pair copulations often begin to take place much earlier than the onset of egg laying may indicate that, in addition to serving as a paternity guard, they play a certain role in pair bonding and perhaps provide a display of the general quality of the male (Lens et al. 1997).

The relatively low rate of in-pair copulations in the Semipalmated Plover is similar to rates observed in some other shorebirds (reviewed in Birkhead et al. 1987). My results provide some (albeit weak) indirect support for three not necessarily mutually exclusive suggestions: (1) male Semipalmated Plovers mostly rely on guarding/territorial defense as paternity guards, (2) copulations may be too costly and thus have to be placed strategically and (3) the need to retaliate arises infrequently. A similar percentage of EPCs (6.7%) to that found in Semipalmated Plovers was also detected in the Lesser Kestrel *Falco naumanni* (Negro et al. 1992) and these copulations also resulted in a very low EPP rate (3.4%) (Negro et al. 1996). The rates in Semipalmated Plovers are

also very similar to those detected in the European Oystercatcher *Haematopus ostralegus* (5% EPC% and 1.5% EPP) - the only other monogamous shorebird whose paternity has been examined using a molecular genetics technique (Heg et al. 1993).

High breeding synchrony. Explanations of the impact of breeding synchrony on EPP rates in birds are somewhat controversial. One hypothesis stipulates that with high breeding synchrony EPC and corresponding EPP rates should be lower since males with fertile mates would have to trade mate-guarding for extra-pair copulations (Birkhead & Biggins 1987, Westneat et al. 1990). Stutchbury and Morton (1995) at the same time argued that an increase in breeding synchrony will be associated with a higher EPP rate and provided evidence from a wide range of species supporting their hypothesis. The authors suggested that at higher breeding synchrony females will have a better chance of assessing the quality of competing males while males potentially will have access to a greater number of fertile females. The study (Stutchbury & Morton 1995) specifically assumed that net benefits from extra-pair activities to both sexes will be higher than costs. However, it only addressed the situation in songbirds where partner investment in broods is often asymmetrical (i.e., males rarely contribute

to incubation) and provisioning by males may not be crucial for survival of the young. In the Semipalmated Plover involvement of both parents in incubation and brood rearing is important (Sullivan Blanken 1996). This may provide an explanation for the fact that the case of extra-pair paternity was observed in the year with lower breeding synchrony. In 1996 more opportunities for extra-pair interactions may have existed as some males could have already nested and thus did not need to guard their own mates as frequently while others could be still searching for a mate and thus attempting to attract any female and/or copulate with her. Therefore data collected during this study support Westneat's *et al.* (1990) hypothesis as (1) the overall breeding synchrony in the Semipalmated Plover is high while the EPP rate is low in comparison to other species (Birkhead & Møller 1995, Stutchbury & Morton 1995) and (2) the incidence of extra-pair paternity was observed in the year with lower breeding synchrony. High breeding synchrony in the Semipalmated Plover, in general, may indeed create favourable conditions for extra-pair mate assessment as maintained by Stutchbury and Morton (1995) and this may be one reason why some extra-pair events do take place (Wagner 1991). Mate sampling was thought to be a plausible explanation for existence of EPCs in the European

Oystercatcher (Heg et al. 1993). In the Semipalmated Plover, however, females may have an opportunity to use this information only during the next breeding season. This is because (1) the length of a given breeding season is too short to adjust mate choice through mate switching as is known to occur in the Kentish Plover *Charadrius alexandrinus*, a temperate region species (Székely & Lessells 1993) without jeopardizing the entire breeding effort. (2) Mate-guarding by males may prevent females from successfully engaging in EPCs and thus adjusting their mate choice via indirect means (Møller & Birkhead 1993a, Stutchbury et al. 1994). The fertilization assurance hypothesis does not appear to have support in this study because (1) no unhatched eggs could be unambiguously classified as unfertilized and (2) the male that lost paternity in 1996 successfully fathered a brood in 1997.

High breeding synchrony is one of the results of a short breeding season. The short breeding season typical for the Arctic/Sub-Arctic climatic zone results in a brief pre-laying/courtship period, the absence of second clutches and very few renests in the Semipalmated Plover population near Churchill. In these conditions any considerable investment of time and energy in EPCs may result in a damage to or loss of individual's own reproductive effort as later

nesting pairs may not have enough time to successfully complete their breeding or renest should the first clutch be lost.

Open nesting habitat. Degree of openness of nesting habitat may play an important role in extra-pair behaviours in birds (Westneat et al. 1990). This factor may have drastically different effects on EPP rates in birds depending on the balance of costs and benefits EPP provides to males and females (Westneat et al. 1990). Many paternity studies conducted to date (e.g. Stutchbury et al. 1994, Yezerinac et al. 1995, Lens et al. 1997) involved small passerine birds occupying visually occluded, woodland or scrubland type habitats which create favourable conditions for stealthy extra-pair males and/or females seeking extra-pair copulations. Three recent studies of monogamous passerines (Bjørnstad & Lifjeld 1997, Fridolfsson et al. 1997, and Reyer et al. 1997) nesting in open alpine type habitats and breeding relatively synchronously revealed EPP rates of 33 and 28% in the Willow Warbler *Phylloscopus trochilus* and 5.2% in the Water Pipit *Anthus spinoletta*. These results provide interesting material for comparison with the present work. Despite the generally open habitat cited in the Willow Warbler studies, this little insectivorous bird is mostly a foliage feeder inhabiting

scrub/tall grass patches even in generally open areas (reviewed in Cramp 1988). Therefore the specific environment where adult individuals interact can probably not be qualified as open. This means that conditions in which either extra-pair males can enter territories with fertile females or females can escape guarding of their mates are present. The Water Pipit, on the other hand, inhabits grassy slopes and meadows and is a ground feeder (reviewed in Cramp 1992). Thus, the general structure of the habitat that this bird occupies is very similar to the one used by Semipalmated Plovers and therefore this factor (degree of openness) may be a reason for generally low EPP rates in these two species. The same reasoning may also apply to the case in the European Oystercatcher, where these birds nested on a saltmarsh and foraged on open beaches.

High return rates. High return rates and presumed familiarity with many individuals breeding in the area from the previous breeding season(s) may decrease females need for correction of their mate choice with EPCs (Ritchison et al. 1994). This is because they may choose or reject old mates based on the previously collected information. This 'historical' information is rarely available in passerine birds with short life-spans often displaying high EPP rates. The low EPC/EPP rate may also go in accord with high divorce

rates (21%) (Flynn et al., in press). In the Semipalmated Plover on average 59% of adult males and 41% of adult females return to the area to breed in the following year (Flynn et al., in press). An additional factor that may reduce the need to correct mate choice via EPCs is that in some small shorebirds mating may start at stop-over sites prior to their arrival to the breeding ground (Frodin et al. 1994), although this is not known specifically for the Semipalmated Plover. Songbirds are not known to use stop-over sites for any considerable period of time nor do they migrate in clearly defined flocks consisting of a relatively permanent number of members which may result in mutual unfamiliarity of birds that settle to breed together, relative to the total pool of breeders in the area. Thus, a need to correct mate choice may arise more frequently in small songbirds than in longer living monogamous species with high return rates.

In this study all young in the brood were fathered by an extra-pair male. Thus the observed distribution of extra-pair young in Semipalmated Plover families appears to be bimodal (none or many). This pattern reported in other studies is usually linked to selective copulations by the female with extra-pair males when they seek genotypic benefits (e.g. Kempenaers et al. 1992, 1997). The random

distribution of extra pair young occurs when the hypothesized fertilization assurance and/or genetic diversification of offspring in broods can be suspected. Even though in general the EPP pattern observed in this study seems to be similar to those reported for species where females were thought to receive certain indirect (genotypic) benefits from EPCs, the overall scarcity of extra-pair young in the population implies that a mixed reproductive strategy can only be rarely pursued by either sex.

Overall the EPP rate in the Semipalmated Plover is low indicating that despite the confirmation that extra-pair copulations do result in fertilizations, Semipalmated Plovers largely maintain socially as well as genetically monogamous bonds between members of a pair. Therefore the observed reproductive (fledging) success may serve as a good indicator of realized reproductive success in the species (e.g. Weatherhead & Boag 1997).

CONCLUSIONS

This study provides the first genetic insights into the true mating system of an Arctic-breeding monogamous shorebird.

The general contribution of extra-pair copulations to sexual selection in the Semipalmated Plover population residing near Churchill, Manitoba, Canada appears to be low as male social partners have been shown to sire, on average, over 95% of the offspring in their families. In at least one situation, however, the potential for EPP was realized since extra-pair young were produced. Costs associated with an active engagement in extra-pair events seemed to be higher than the potential benefits for either males or females. It, therefore, can be stated that a mixed reproductive strategy is either not actively pursued or cannot be efficiently realized in the Semipalmated Plover and the genetic make up of the offspring is largely a true reflection of observed social monogamy.

This study did demonstrate clearly that extra-pair paternity occurs in at least some populations of the Semipalmated Plover. The general circumstances of this occurrence, however, seem to indicate that may be more of a reflection of a chance combination of temporal (i.e., synchrony) and spatial (i.e. density) distribution of

breeding birds (Reyer et al. 1997) than a 'concentrated effort' in pursuing a mixed reproductive strategy by either sex.

EPCs occurred at a rather low rate in the Semipalmated Plover and this resulted in equally low EPP frequency. With the absence of a strong direct impact on realized reproductive success of individuals EPCs may serve a different purpose. They may provide females with information on male quality and this information can be used in subsequent breeding attempts.

Additional work will provide many new interesting details about still unknown aspects of mate choice and breeding behaviour in the species. It is, however, unlikely that a noticeably different situation with paternity will be discerned in other populations of the Semipalmated Plover breeding in those Arctic/Sub-Arctic environments where similar environmental conditions exist. A broader comparison, however, encompassing temperate, sub-Arctic and high Arctic populations of the species will undoubtedly yield very interesting results. This study may serve as a first step for such a comparison.

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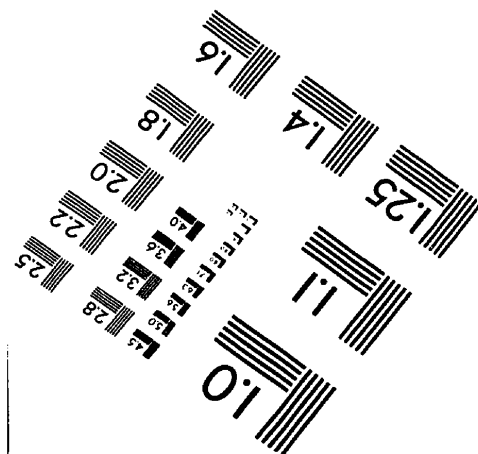
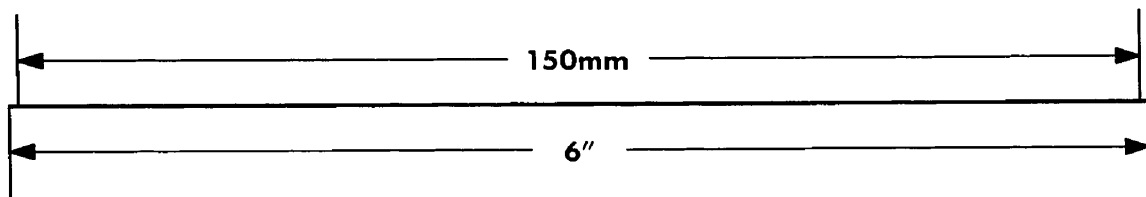
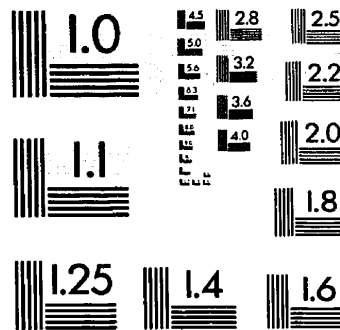
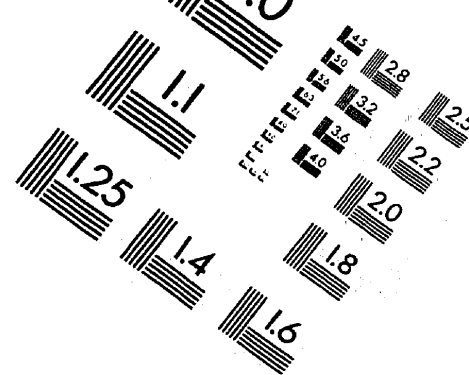
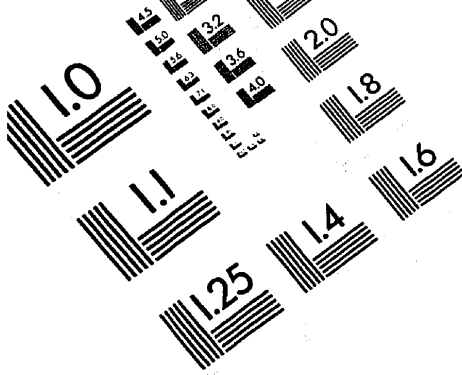
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Appendix 1. Mating systems and occurrence of EPCs and EPP in five shorebird species for which DNA fingerprinting has been conducted.

Species	Mating system	EPC (%)	EPP (%)	Source
Oystercatcher, <i>Haematopus ostralegus</i>	monogamy	10-15	1.5	Heg et al. 1993
Dotterel, <i>Charadrius morinellus</i>	polyandry	-	4.6	Owens et al. 1995
Semipalmated Plover, <i>Charadrius semipalmatus</i>	monogamy	7.1	4.7	this study
Spotted Sandpiper, <i>Actitis macularia</i>	sequential polyandry	11.4	2.9-14.3	Oring et al. 1992
Buff-breasted Sandpiper ¹ , <i>Tryngites subruficollis</i>	lekking	7	40	Lanctot et al. 1997

1. For the Buff-breasted Sandpiper percentages of females visiting multiple leks and brood with multiple paternity respectively are given.



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