

Secondary Reproductive Strategies in Pacific Black Brant

(*Branta bernicla nigricans*).

by

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THESIS

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by

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## Abstract

Waterfowl are known to use secondary reproductive strategies, both extra-pair copulations and intraspecific brood parasitism, to increase fitness. We used five polymorphic microsatellite loci to determine extra-pair paternity and nest parasitism in 30 nests of Pacific Black Brant geese (*Branta bernicla nigricans*) containing 108 offspring. Fourteen of the 30 nests contained offspring that were not genetically related to one or both of the attending adults: 6.5% (7/108) of the offspring resulted from extra-pair copulations (EPC); 13.9% (15/108) of the offspring resulted from intraspecific brood parasitism (IBP). All offspring resulting from EPCs were produced during the peak period of nest initiation. Adult females hosting parasitic eggs were significantly older than non-hosts. After accounting for eggs resulting from IBPs in the calculated clutch size, clutches containing IBPs were significantly smaller than unparasitized clutches. Our data indicate that secondary strategies represent an important component of reproductive effort in Black Brant.

## Table of Contents

<b>Abstract</b> .....	3
<b>Table of Contents</b> .....	4
<b>List of Tables and Figures</b> .....	5
<b>Acknowledgments</b> .....	6
<b>Introduction</b> .....	7
<b>Materials and Methods</b> .....	12
<b>STUDY SITE AND SAMPLING STRATEGY</b> .....	12
<b>LABORATORY ANALYSIS</b> .....	13
<b>STATISTICAL ANALYSIS</b> .....	16
<b>Results</b> .....	17
<b>Discussion</b> .....	19
<b>POTENTIAL TECHNICAL ERRORS</b> .....	19
<b>EXTRA-PAIR COPULATIONS</b> .....	20
<b>INTRASPECIFIC BROOD PARASITISM</b> .....	23
<b>COMPARISONS WITH STUDIES OF OTHER GEESE:</b> .....	25
<b>References</b> .....	26

## List of Tables and Figures

Table 1. Statistical summary of the 5 loci.....	34
Table 2. Offspring alleles that were inconsistent with those of the attending adults.....	35
Figure 1. Allele frequencies at 5 microsatellite loci in Pacific Black Brant ( <i>Branta bernicla nigricans</i> ).....	36
Figure 2. Estimates of incidence of extra-pair copulations (EPCs) and intraspecific brood parasitism (EBP) relative to total nests.....	37
Figure 3. Temporal patterns of extra-pair copulations and intraspecific brood parasitism.....	38

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## Introduction

Until recently, the primary mating system of most avian species (96%) was thought to be monogamy (Lack 1968). Monogamy is beneficial to species with prolonged parental care; it reduces energy and time expenditure associated with courtship, infertility due to physical incompatibility, and decreased fecundity associated with inexperience by one member of a pair (McKinney 1992). Male and female waterfowl commonly use secondary reproductive strategies to increase their fitness (McKinney 1986). Male strategies were originally thought to be limited to mate acquisition and territory defense. However, Trivers (1972) predicted that males could benefit by soliciting copulations outside of the pair bond. Behavioral observations (McKinney et al. 1983) demonstrated that unpaired individuals engaged in extra-pair copulations (hereafter "EPCs"), and Cheng et al. (1982) provided evidence that such copulations resulted in fertilized eggs, indicating that EPCs represent a viable secondary reproductive strategy for monogamous, primarily temperate breeding, ducks (tribe Anatini).

Absence of resource-based polygyny in waterfowl, as predicted for mating systems in species with precocial young (Emlen and Oring 1977), suggests that female waterfowl influence the mating system (McKinney 1986, Rohwer and Anderson 1988). In ducks, a female biased sex ratio forces males to pair monogamously long before the breeding season (Rohwer and Anderson 1988). However, abandonment of their mates following egg laying, and asynchronous nesting provides most male ducks the opportunity to use a "mixed reproductive strategy" (Trivers 1972, Afton 1983, Cheng et al. 1982). Studies

using captive mallards (*Anas platyrhynchos*) indicate that males effectively increase their fitness by targeting forced copulations during a female's "fertility window" (Cheng et al. 1982, Cheng et al. 1983).

Mating systems of high latitude waterfowl, in contrast to those in temperate areas, are influenced by the short arctic breeding season that constrains both pair-bond formation and nutrient stores required by females for breeding. Unlike ducks, male geese contribute to parental care after hatch by maintaining long-term pair bonds (Sedinger et al. 1995) and male defense of feeding areas may be necessary to allow females to store nutrients before egg laying (McLandress and Raveling 1981). Arctic geese nest highly synchronously; >70% of nests may be initiated in less than 10 days (Cooke et al. 1995). Thus, male geese may be limited in opportunities to seek extra-pair copulations by this highly synchronous breeding and the need to guard their mates.

Opportunities for EPCs are influenced by nesting density, synchrony, and mate guarding (Westneat et al. 1990). For males, not constrained by the nutritional requirements of egg production or incubation, factors increasing opportunities for EPCs can increase fitness. Potential benefits to males of seeking EPCs seem clear, but males pursuing EPCs may suffer a cost of cuckoldry because unguarded females may be subjected to a higher frequency of EPC attempts (Lamprecht 1989). Higher nest density enables males to pursue EPCs from neighboring females, yet retain proximity for mate defense if necessary (Sorenson 1994, Lank et al. 1989a). The conflict between the benefits of seeking EPCs and the costs of increased risk to being cuckolded should favor

males that wait until their own mate has completed laying before seeking EPCs.

Asynchrony in nesting should favor males whose mates nest relatively early so the females complete laying and are no longer fertile before most other females initiate laying. Such males would be free to seek EPCs during most of the egg-laying period without risk of being cuckolded. Under such a strategy we would expect EPCs to occur during the peak egg-laying period when early nesting males are less constrained by mate guarding.

Male opportunities for successful EPCs are strongly affected by female behavior (Westneat et al. 1990, Birkhead and Moller 1993). Behavioral observations indicate that female birds generally respond to EPCs in one of three ways: 1) active solicitation, 2) apparent resistance, or 3) passive acceptance (Westneat et al. 1990). Females participating in active solicitation initiate visual or vocal contact with extra-pair males (Westneat et al. 1990). However, this behavior may not be beneficial to female arctic geese that are constrained by limited time to uptake nutrients prior to egg laying (Raveling 1978). Female waterfowl have been observed avoiding forced copulations (McKinney et al. 1983, Welsh and Sedinger 1990); they may be attempting to avoid physical injury, fertilization by poorer quality males, reduced parental care (McKinney and Evarts 1998) or intestinal parasites (Moller 1994, but see Lombardo et al. 1999). Females in many avian species appear to accept or cooperate with EPCs initiated by males (Stutchbury and Neudorf 1998). The advantage of long term pair bonds and associated low incidence of “divorce” among geese (Forslund and Larsson 1991) minimizes the costs of EPC acceptance for females because males are not likely to seek alternative mates. Benefits of

participation in EPCs potentially include ensured fertilization of eggs or the proliferation of “good genes” (Liffield et al. 1993, Strohbach et al. 1998).

Females also may use intraspecific brood parasitism (hereafter, “IBP”) as part of a mixed reproductive strategy to reduce costly parental care, spread risk of total brood loss among nests, or reduce competition among siblings (Petrie and Moller 1991). Among arctic nesting geese, physiologically constrained in egg laying by nutrient reserves, IPB is thought to be used largely as a “salvage” tactic by females that have a low likelihood of nesting successfully (Lank et al. 1989b). For avian species that lack post-hatch parental care, there may be little evolutionary disadvantage to being parasitized, so evolutionary pressures to defend against IBP are weak (Sayler 1992). The close proximity of individuals nesting colonially is predicted to facilitate IBP in waterfowl (Sayler 1992). Furthermore, IBP enables females without adequate resources for both egg production and incubation to breed (Oring and Sayler 1992).

Hosts of parasitic eggs may benefit by diluting predation risk to the adults’ own offspring (Nudds 1980, Eadie and Lumsden 1985). Larger brood size resulting from IBP may provide advantages in social encounters while foraging during brood rearing (Gregoire 1985) or on the wintering grounds (Raveling 1970, Choudhury et al. 1993). Clutch size enlarged by IBP could negatively affect hatching success because females may extend their incubation period past the time when their own goslings hatch if parasitic eggs are laid after a female’s own clutch is complete (Lank et al. 1989b, Sedinger 1986, Eichholz and Sedinger 1998). However, mechanisms increasing synchrony (MacCluskie

et al. 1997) may reduce variation in timing of hatch associated with IBP. Increased brood size resulting from IBP may influence subsequent reproduction as Lessells (1986) found that female Canada Geese delayed nesting in the year following artificial brood augmentation.

Numerous studies incorporating molecular techniques (primarily DNA fingerprinting) have documented EPCs (Birkhead and Moller 1992). Although large conceptual advances in our understanding of avian mating systems were initially made by waterfowl biologists (Oring and Saylor 1992, McKinney 1992, Rohwer and Anderson 1988), few paternity studies using genetic markers have been conducted on wild waterfowl populations. Difficulty in sampling adults, and small clutch sizes from which to derive paternity limited the applications of DNA minisatellites to many waterfowl species. Microsatellites have enhanced studies of kinship (Queller et al. 1993) because the polymerase chain reaction (PCR) can be used to amplify DNA from feathers and eggshell membranes (Pearce et al. 1997). For this study, we used microsatellites to evaluate the fitness consequences of extra-pair fertilizations and intraspecific nest parasitism in arctic breeding Pacific Black Brant (*Branta bernicla nigricans*) (hereafter, brant) geese. In particular, we examined temporal variation in EPCs with respect to the pattern of nest initiation, and variation in age and clutch size of hosts for parasitic eggs.

## Materials and Methods

### *Study site and sampling strategy*

This study was conducted on the brant colony at Tutakoke (61°15'N, 165°37'W) located within the Yukon Delta National Wildlife Refuge in western Alaska. The brant colony occupies approximately 5 km<sup>2</sup> along the Bering Sea coastline. Topography is flat with less than 0.5 m of vertical relief in terrestrial sites throughout the colony (Jorgenson and Ely, in press). Nesting occurs in wet sedge and grass meadows extending up to 2 km inland from the intertidal mudflats, and on islands in numerous small ponds.

Brant have been studied continuously at the Tutakoke Brant Colony since 1984, and approximately 2,000 Brant have been marked annually since 1987. During adult remigial molt, banding 'drives' are used to herd from ca. 100 to 2000 brant into corral traps on brood-rearing areas. Brant are marked with uniquely coded alfa-numeric plastic leg bands in addition to metal USFWS bands (Sedinger et al. 1997). Annual probability of retaining a plastic leg band was 0.998 (Ward et al. 1997), resulting in about 30% of the ca. 5,000 nesting pairs at Tutakoke being individually identifiable.

Regular searches for nests associated with at least one marked adult were conducted from nest initiation through hatch in 1996 and 1997. Throughout egg laying we located nests by searching 50 m radius plots. During incubation and hatch, when females were more tenacious to nest sites, we also located nests opportunistically by flushing females and examining them for leg bands. We read bands on males defending the nest site, if

present, or the male that joined the female after she left the nest. Nests were assigned a unique nest number, and eggs were marked with a permanent marker to identify their nest and position in the laying sequence. If more than one new egg was present in a nest during a particular visit, laying sequence was often established by the degree of shell staining on each egg; first eggs being the most stained (Flint and Sedinger 1992). Multiple observers verified most adult tarsus bands associated with specific nests.

We visited nests at hatch to acquire tissues from nesting females and offspring from that nest. We collected contour feathers that females had used to line their nest bowls as a sample of female tissue. Contour feathers were stored dry in stationary envelopes or plastic baggies. We collected egg membranes from nests at hatch to provide tissue samples from offspring hatched in that nest. Growing feathers were collected from all banded males recaptured during banding drives. Vascular portions of the eggshell membranes and growing feathers were stored in individual vials containing 70% ethanol.

### *Laboratory analysis*

Various microsatellite loci from white-fronted geese (*Anser albifrons*), tundra swans (*Cygnus columbianus*), harlequin ducks (*Histrionicus histrionicus*), and Canada geese (*Branta canadensis*) (Fields and Scribner 1996, Buchholz et al. 1998, Cathey et al. 1998) were tested for variability in Black Brant. Five loci designed from Canada goose DNA were used in this study: Bca1, Bca11, Bca7, Bca4 (Buchholz et al. 1998) and 5AB (Cathey et al. 1998). Primers for these loci were redesigned with an ABI™ compatible fluorochrome (TET, FAM, or HEX) and re-optimized for PCR. PCR parameters for

[ $\alpha^{32}\text{P}$ ]-ATP primers did not require significant alteration from published parameters (Buchholz et al. 1998, Cathey et al. 1998) after fluorochrome labeling, although AmpliTaq Gold™ (PE- ABI) improved PCR amplification (decreased “stutter” bands) for primers Bca1, Bca11, and Bca4. Plus A, an artifact of PCR, erratically results from the tendency of Taq polymerase to add a non-templated nucleotide (usually an A) to the 3' end of double stranded DNA (Clark 1988). To remediate this problem, PCR amplifications were forced to PlusA with a 45 minute annealing temperature extension at the end of the PCR program.

Samples were analyzed in the laboratory approximately 18 months following field preparation. Tissue samples of 653 males were collected at banding, and matched with associated mates and offspring from nest samples. As a result, we had complete sets of tissues for thirty families from 1996 (n=3), 1997 (n=23), 1996 and 1997 (n=2) that were analyzed for paternity. Two pairs of adults bred and were sampled in both 1996 and 1997. DNA from the three tissue types (contour feathers from the nests, male feathers, and egg shell membranes) was extracted using Quiagen extraction kits (Quiagen 1996). DNA was quantified with a spectrophotometer, and amplified using 150 ng DNA per 10  $\mu\text{L}$  PCR reaction.

To determine allele sizes of the five loci, PCR products were electrophoresed on an ABI Prism™ 373 Automated Sequencer (ABI). Lanes were co-loaded with three PCR products (distinguished by different dye labels) and an internal lane standard, TAMRA350 (ABI). A standard individual (#722) was run on each gel to ensure consistency.

GeneScan™ and Genotyper™ software (ABI) were used to analyze and archive data. Alleles scores for each locus were classified (ca. +/- 1 bp) to the nearest allele group, however, relatedness was determined by both allele and peak comparisons (Schwengel et al. 1994). Individuals concluded to be the result of extra-pair paternity (EPC or IBP) were reanalyzed to confirm results. Offspring genotypes not consistent with the genotype of the attending male at any locus were classified as resulting from extra-pair copulations. Offspring with alleles not consistent with either the maternal or both of the attending adults at any locus were considered to have resulted from IBP.

We used Genepop3.1 (Raymond & Rousset 1995a, 1995b) to estimate allele frequencies, test each locus for Hardy-Weinberg equilibrium, and to test loci pairs for linkage disequilibrium. Fishers Exact test was used for paired locus disequilibrium tests; Bonferroni correction was used to adjust the significance level of the linkage disequilibrium test for the total number of pairs tested (Weir 1990). Exclusion probability was the probability by which we could exclude an attending parent as a genetic parent of an offspring when in fact that parent was not the genetic parent. We calculated the exclusion probability  $Q_i$ , the probability by which we could detect an incorrectly assigned parent at a single locus as:

$$Q_i = \sum_u p_u(1-p_u)^2 - \frac{1}{2} \sum_u \sum_{v \neq u} p_u^2 p_v^2 (4-3p_u-3p_v) \quad (1)$$

where  $p_u$  = allele frequency of the  $u$ th allele.

The combined overall probability of exclusion,  $Q$ , for multiple loci was

$$Q = 1 - \prod_1 (1 - Q_i) \quad (2)$$

(Weir 1990).

The probability of identity, the probability that two individuals in a population shared a genotype by chance, for a locus was

$$\sum_{i=1}^n (q_i^2)^2 + \sum_{\substack{i,j=1 \\ i>j}}^n (2q_i q_j)^2, \quad (3)$$

where  $q_i$  was the frequency of the  $i$ th of  $n$  alleles.

The combined probability of identity, using all loci, was the product of the probabilities at each locus

$$\prod_{k=1}^m \left[ \sum_{i=1}^n (q_i^2)^2 + \sum_{\substack{i,j=1 \\ i>j}}^n (2q_i q_j)^2 \right] \quad (4)$$

(Hanotte et al. 1991).

### *Statistical Analysis*

For nests located during egg laying, we estimated the date of nest initiation by back-dating from the date the nest was located, assuming that one egg was laid per day with a day skipped between eggs 4 and 5 (MacInnes 1962). Initiation dates for nests located during incubation were back-calculated from the hatch date, assuming a 23 day incubation period (Eichholz and Sedinger 1998). We classified sample nests as early laying, peak

laying, or late laying based on their relationship to the overall pattern of nest initiation within the colony prior to statistical analysis. 'Early laying' represented the four day period when approximately the first 15% of nests were initiated, while 70% of the nests were initiated during the five days of 'peak initiation'. 'Late laying' was the four day period when the last 15% of nests were initiated. We used a  $\chi^2$  contingency table to test for associations between timing of nesting and frequency of both EPCs and IBPs.

Adult ages at time of genetic sampling were estimated from banding data. Most adults had been banded as adults, indicating they were at least two years old when banded (Bellrose 1980). We could only estimate minimum ages of these individuals by assuming they were a minimum of two years old when they were originally banded. A Student's *t*-test was used to evaluate age differences of birds with and without offspring resulting from IBPs or EPCs in their nests. Similar analysis was conducted to determine if clutch size (after subtracting eggs resulting from IBPs) varied significantly between nests with and without IBP eggs.

## Results

The five microsatellite loci displayed from 3 to 19 alleles per locus in brant. Allele frequencies ranged from 0.009 to 0.96 with 73% of all alleles having frequencies <10% (Figure 1). Statistics for loci were based on the genotypes of 56 adults. Locus Bca4 demonstrated Z chromosome-linkage as previously suspected (Buchholz et al. 1998), contributing to overall heterozygote deficiency ( $P = 0.0135$ ) (Table 1). Allele frequencies

were within expectations of Hardy-Weinberg equilibrium ( $P > 0.05$ ). The Markov chain test indicated no linkage disequilibrium ( $P > 0.005$ ). The overall probability of detecting an incorrectly assigned parent, or the exclusion probability, was 0.96 for females and 0.97 for males. The probability that two individuals in the population would share a genotype across all loci by chance was  $3.5 \times 10^{-4}$  for females and  $1.41 \times 10^{-4}$  for males (Table 1).

Fourteen of the 30 nests (47%) contained offspring that were not related to at least one of the attending adults. Twenty one percent of all offspring (23 of 108) analyzed were the result of either extra-pair copulations (EPCs) or intraspecific brood parasitism (IBP) (Table 2). Thus, 6.5% (7 of 108) of the offspring were the result of extra-pair copulations; 13.9% (15 of 108) of all offspring were the results of IBP. One egg fitting the criteria for IBP was the first egg laid in the nest, and, thus may have represented usurpation of the territory by a second pair of birds. The genotype of one offspring (0.93%) was ambiguous due to the aberrant allele being associated with the sex-linked locus, *Bca4* (Table 2). Without sexing the individual, it was not possible to determine if the offspring was homozygous for the aberrant allele, or not related to either adult. The proportion of eggs per nest resulting from extra-pair paternity varied; one nest had all its offspring resulting from EPC, four nests contained a single extra-pair young that resulted from IBP, and two nests contained eggs resulting from both EPC and IBP (Figure 2). Of the two adult pairs tested both years, nest PAS015 was the only nest associated with extra-pair paternity (clutch size  $n=5$ ), having one EPC and one IBP in 1996.

All nests containing EPCs fell nonrandomly within 2 days of peak initiation (ca. 75% of all nests were initiated within this period), although the sample size ( $n = 4$ ) was not large enough to detect a significant temporal pattern ( $\chi^2 = 3.308$ ;  $df = 2$ ;  $P = 0.136$ ). IBPs did not demonstrate a temporal pattern (Figure 3). Student's  $t$ -test, however, indicated that ages were significantly higher for females with parasitic eggs ( $t = 2.14$ ;  $df = 21$ ;  $P = 0.03$ ) than for females with no IBP. Clutch size of host females was significantly smaller for nests containing IBPs ( $t = 9.50$ ;  $df = 23$ ;  $P < 0.001$ ).

## Discussion

### *Potential technical errors*

Use of the polymerase chain reaction can result in errors of quantification and genotyping associated with contamination, null alleles, and mutation (Taberlet et al. 1999, Fitzsimmons 1998, Jones et al. 1998). The short amplification products of microsatellites are amplified regardless of poor quality DNA, and low quality DNA is susceptible to contamination (Taberlet et al. 1999). This problem can occur in field conditions where persons collecting samples are not trained to minimize the risk of DNA contamination. In this study, potential contamination issues were minimized because one person oversaw the entire process from sample collection in the field to assessment of electrophoretic peaks. Double banding indicative of sample contamination (Pearce et al. 1997, L. Davis pers. comm.) was not observed for any of the samples.

Low exclusion rates, null alleles and mutations are recognized as confounding factors in microsatellite kinship studies. The exclusion and identity probabilities observed here ( $3.5 \times 10^{-4}$  and 0.96, respectively for females) are lower than demonstrated in some other studies (e.g. Fitzsimmons 1998). We acknowledge our estimates of EPC and IBP rates may be conservative. Null alleles produce no discernible product and result in false homozygotes. Mutations may arise from repeat length variation due to slipstrand mispairing during replication, and result in alleles distinct from parental genotypes (Taberlet et al. 1999). Although the loci we used have also been used in several other population studies without detecting mutation (Pierson et al. in press), we recognize that this phenomenon might have caused us to overestimate rates of EPCs or IBP. In our data, 11 of the 23 extra-pair offspring were classified as unrelated to parental genotypes based on a single locus for which they were homozygous. If null alleles are indeed commonplace (Pemberton et al. 1995), our data still provide genetic evidence for extra-pair paternity and brood parasitism, however at a lower rate (2/108 offspring resulting from EPC, 9/108 offspring resulting from IBP).

### *Extra-pair copulations*

Our results provide unambiguous evidence that extra-pair copulations serve as a secondary reproductive strategy for brant. Fertilizations resulting from extra-pair copulations (6.5% of all offspring) documented in this study were lower than expected from behavioral observations (Welsh and Sedinger 1990, see Dunn and Liffield 1994). Welsh and Sedinger (1990) estimated an average EPC rate of 0.8/pair/day, and estimated

25% of all copulations were EPCs. Our data indicate that approximately 1 in 4 (0.065/0.25) EPCs result in fertilization. The contrast between EPC rate and resulting fertilization suggests that extra-pair males cannot use copulations as a reliable indicator of their share of paternity (Lifjeld et al. 1994).

Seventy percent of the population of Pacific Black Brant nest in four major colonies on the Yukon-Kuskokwim Delta containing 3000-7000 nests each (Anthony et al. 1995). Colonial nesting among Brant is assumed to offer the advantage of reduced predation by Arctic foxes through predator swamping (Raveling 1989). Nesting in higher densities, however, may increase variation in male reproductive success. High density nesting clearly offers males increased proximity to females relative to dispersed nesting. Males nesting at high density that are socially dominant may increase the number of copulations they engage in, thus, increasing the number of eggs they fertilize relative to males nesting at lower densities. Less dominant males, in contrast, may experience reduced paternity. Overall fitness advantages of reduced predation risk to males nesting at high density may be enhanced or offset by EPCs, depending on social status of the male.

Brant reproductive effort is synchronous; the majority of birds initiate nests within 5-7 days in most years (Lindberg and Sedinger 1997). Upon arriving on the breeding grounds, males face a tradeoff between mate guarding and leaving their mates unattended to seek EPCs from fertile females. Male brant expend substantial energy guarding their mates during nest initiation (Welsh 1988) when the risk of being cuckolded is greatest. Once a male's mate has completed egg-laying and is no longer fertile, the cost of his

seeking EPCs is substantially reduced. We expect that this asymmetry leads to variation in the temporal patterns of EPCs. Males associated with nests initiated relatively early will be freed from mate guarding when the majority of females are initiating their nests. Our data, consistent with this hypothesis, indicate that fertilization of eggs by EPCs does not occur during the early nest initiation period, but is focussed during the peak of colony-wide nest initiation. We also did not observe eggs fertilized by EPCs during the late nest initiation period when only about 19% of females were still laying and, thus, fertilizable. Opportunities for EPCs for individual males were reduced during late nest initiation, which could have reduced the proportion of males seeking EPCs. Thus, our findings suggest a threshold for males soliciting EPCs (or females accepting them) that may correspond with the significant decline of fertilizable females relative to spatial availability. Alternatively, the probability we happened to sample an egg fertilized by an EPC was low for nests initiated late and our failure to detect EPCs in “late” initiated nests could have been due to chance alone.

The importance of investing time and energy in nutrient uptake before egg laying (Sedinger et al. 1993, Flint and Sedinger 1992) and beginning incubation with laying of the second egg has likely restricted brant from evolving a mating system in which females actively solicit EPCs (see Davies 1992). Behavioral observations (P. Svete personal obs., Welsh 1988, Welsh and Sedinger 1990) indicate that female participation in EPCs is limited to forced copulations in the absence of pre-copulatory behavior, and passive participation in EPCs from soliciting males. Females may, nevertheless, be motivated to

participate in EPCs if they provide an opportunity to mate with phenotypically superior males (Yezerinac and Weatherhead 1997). Low divorce rates (Sedinger et al. unpubl., Cooke et al. 1995) among brant may limit the “pool” from which females could pick a new mate if they were paired to a low quality male, limiting their options to form new pair bonds (Forslund and Larsson 1991, J. Sedinger pers. obs.). Thus, female brant may consent to solicited EPCs to obtain “good” genes (Weatherhead and Roberson 1979). Females may use the size of males’ white neck collar as an indicator of male age and rank (Abraham et al. 1983, Lindberg and Sedinger 1997, Moller 1994) for assortative extra-pair matings.

### *Intraspecific brood parasitism*

In arctic nesting geese, rapid follicle development commences about the time birds depart from their final migratory staging area (Raveling 1978) due, in part, to benefits of early nesting (Sedinger and Raveling 1986). Waterfowl initiating follicle growth while staging a significant distance from the breeding grounds may be forced to lay eggs before sufficient nest sites are available in late springs, thus promoting IBP (Syroechkovsky 1979). Even though the final spring staging area is within 400 km (one day’s flight time) from brant breeding areas on the Yukon-Kuskokwim Delta, Lindberg et al. (1997) suggested that the increased average clutch size observed when nesting was delayed might be attributed to IBP. Our study confirms that IBPs occur and produce viable offspring. During nest initiation, brant lay one egg approximately every 30-36 hours (D. Person, unpubl. data). The rate of IBP observed in this study (20% of all nests) is consistent with

field observations of supernumerary eggs appearing during daily nest checks (Flint and Sedinger 1992, C. Nicolai pers. comm.).

Previous studies suggest that intraspecific brood parasites may preferentially parasitize hosts that are more experienced, and can provide better care for their young (Eadie 1991, Weigmann and Lamprecht 1991). Because female brant must obtain 80% of the nutrients required for breeding on the nesting grounds before and during egg-laying (Ankney 1984), delays in habitat availability may promote the use IBP as a “salvage” strategy (Lank et al. 1989b) for females unable to obtain the necessary nutrients. Previous studies on this population of brant indicate that older females tend to initiate nests earlier, and have larger clutches (Sedinger et al. 1995). Additionally, in years when nest initiation is delayed at the Tutakoke River colony, mean clutch size is increased and young and small bodied females have a decreased breeding propensity (Eichholz and Sedinger 1999). Our data demonstrated that nests containing parasitized eggs are attended by older females, yet clutch size was significantly smaller for nests containing IBPs than those without. Most nests used for this study were located during hatch, thus lack of enlarged clutches in nests known to contain another female’s eggs could reflect egg loss due to higher rates of depredation. Regardless of clutch size, costs to females hosting IBPs may be minimal during brood rearing because the percentage of time females are alert is not correlated with brood size (Sedinger et al. 1995).

### *Comparisons with studies of other geese:*

Few waterfowl studies have used DNA fingerprinting to test for paternity at the nest (Dunn et al. 1999), avoiding biased estimates due to brood mixing (Larrison et al. 1995) or nonrandom sampling (Quinn et al. 1987). Behavioral observations of lesser snow geese (*Chen caerulescens c.*) and Ross's geese (*Chen rossii*) initiating nests at Karrak Lake, Canada, indicated that 38 and 33% of all successful copulations, respectively, were EPCs (Dunn et al. 1999). However, similar to our findings among brant, only ca. 15-20% of these copulations resulted in fertilization. DNA fingerprinting revealed that 5% of all offspring of lesser snow geese (n=80) resulted from EPC while 1.25% resulted from IBP. For Ross's geese, 2.4% (n= 24) nests contained unrelated offspring resulting from EPCs and no IBPs were detected (Dunn et al. 1999). Contrary to our findings for brant, all extra-pair copulations in lesser snow geese were forced and fertilizations resulting from EPCs occurred when females started nesting relatively early or late (Dunn et al. 1999). Lack of IBP may be a consequence of interspecific heterogeneity resulting from interspersed nesting of Ross's and snow geese within the breeding area. Analysis using plumage markers at La Perouse Bay on a homogeneous nesting population of lesser snow geese demonstrated a higher rate of IBP (5.6%, Lank et al. 1989a) than at Karrak Lake, suggesting that opportunities for IBP may have been reduced at Karrak Lake.

Overall our findings indicate that both EPCs and IBP are commonly used as strategies in brant, consistent with other studies of geese (Lank et al. 1989a, Dunn et al. 1999). Moreover, our data also indicate that the use of these strategies is not random.

Employment of EPCs by males whose mates nested early may enhance their fitness beyond that associated with the early hatch date of their clutches (Sedinger and Raveling 1986, Sedinger and Flint 1991). We are unsure of the identity of parasitic females, but acceptance of parasitic eggs by older females could have enhanced their fitness associated with the advantages of rearing larger broods. Parasites likely benefited from parasitizing older versus younger females.

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**Table 1.** Statistical summary of the 5 loci used to evaluate Pacific Black Brant Paternity.

Observed heterozygosity ( $H_O$ ) and expected heterozygosity ( $H_E$ ) were calculated from a combined pool of male and female genotypes. Calculations for probability of identity and exclusion probability incorporated variation of allele frequencies due to the sex-linked locus, Bca4.

Locus ID	number of alleles	$H_O$	$H_E$	Probability of identity	Exclusion probability
<b>Female</b>					
Bca1	5	0.30	0.29	0.526	0.157
Bca11	4	0.05	0.07	0.866	0.036
Bca4	7	0.00	0.43	0.091	0.573
5AB	19	0.96	0.92	0.015	0.826
Bca7	3	<u>0.41</u>	<u>0.53</u>	<u>0.459</u>	<u>0.293</u>
overall		0.41	0.45	$3.50 \times 10^{-4}$	0.957
<b>Male</b>					
Bca1	5	0.30	0.29	0.526	0.157
Bca11	4	0.05	0.07	0.866	0.036
Bca4	13	0.34	0.43	0.037	0.722
5AB	19	0.96	0.92	0.015	0.826
Bca7	3	<u>0.41</u>	<u>0.53</u>	<u>0.459</u>	<u>0.293</u>
overall		0.41	0.45	$1.41 \times 10^{-4}$	0.972

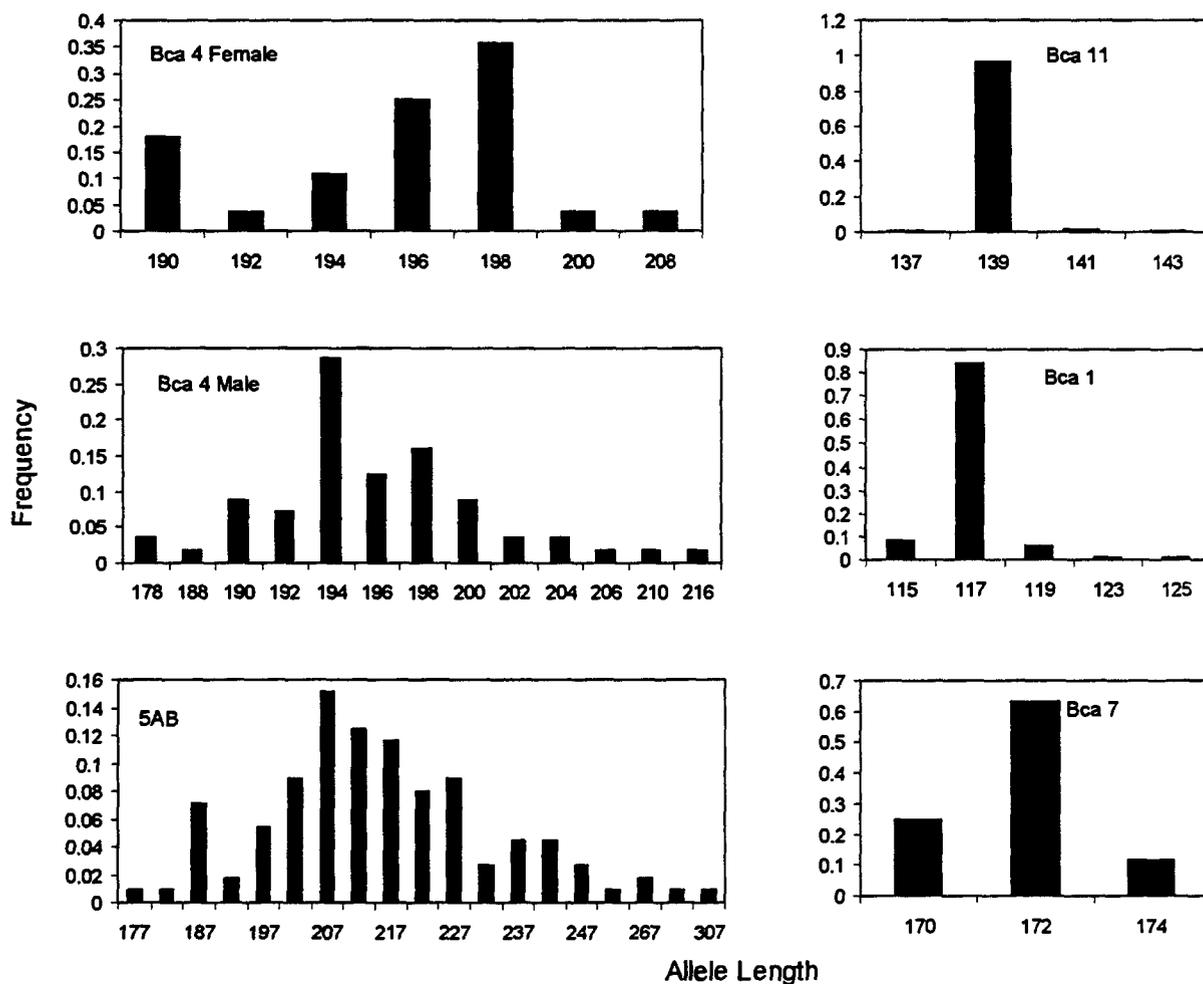
**Table 2.** Offspring alleles that were inconsistent with those of the attending adults.

In the table, alleles are segregated as maternally (F) or paternally (M) inherited. Alleles not associated with the putative parent are bold/underlined. “EGG#” following the number associated with “Offspring ID” refers to the sequence of laying if known for the sample. “E” refers to an egg whose laying sequence is not known. Extra-pair copulations (EPCs) have genotypes not associated with the paternal (or paternal and maternal) allele. Eggs resulting from intraspecific brood parasitism (IBP) have genotypes not associated with the maternal genotype. Offspring “125-EGG1” results from either EPC or IBP. Example: Offspring ‘408-EGG1’, the first egg laid in nest PAS012, had alleles inconsistent with the male at loci Bca4 and 5AB. This offspring is not related to the mother nor father; it results from IBP. Offspring 412-E is also from nest PAS012, but its laying sequence is unknown.

YR	Nest	Offspring ID	Type	Alleles									
				Bca1		Bca11		Bca4		5AB		Bca7	
				F	M	F	M	F	M	F	M	F	M
96	BTP021	838-E	EPC	117	117	139	139	198	194	197	237	174	<b>174</b>
96	PAS015	24-E	EPC	117	117	139	139		200	217	207	174	<b>174</b>
96	PAS015	25-E	IBP	117	117	139	139	<b>188</b>	200	207	207	172	172
96	PAS012	408-EGG1	IBP	115	117	139	139	196	<b>194</b>	<b>242</b>	<b>227</b>	172	170
96	PAS012	412-E	IBP	117	117	139	139		190	212	227	<b>170</b>	170
97	163010	108-E	IBP	117	117	139	139	<b>200</b>	<b>190</b>	217	<b>237</b>	170	172
97	TJO048	709-EGG2	EPC	117	117	139	139		204	222	<b>222</b>	172	<b>174</b>
97	TJO137	728-EGG1	EPC	117	117	139	139	198	196	217	<b>217</b>	170	170
97	TJO137	729-E	EPC	117	117	139	139	198	196	217	<b>217</b>	170	170
97	TJO137	730-E	EPC	117	117	139	139	198	196	217	<b>217</b>	170	170
97	PAS141	733-EGGA	IBP	117	117	139	139	196	190	<b>247</b>	197	172	172
97	PAS141	735-EGGC	IBP	117	117	139	139		190	187	207	<b>174</b>	174
97	PAS141	734-EGGE	IBP	117	117	139	139		198	187	207	<b>174</b>	174
97	DSJ073	738-E	IBP	123	<b>115</b>	139	139		194	207	227	<b>172</b>	172
97	DSJ073	740-E	IBP	<b>125</b>	117	139	139		194	207	227	<b>172</b>	172
97	MJA036	744-E	IBP	117	117	139	139	198	194	<b>187</b>	187	170	172
97	MJA036	746-E	IBP	115	117	139	139		200	<b>202</b>	187	170	172
97	PAS112	806-EGG2	IBP	<b>115</b>	115	139	139	<b>202</b>		207	222	172	172
97	063011	125-EGG1	EPC/IBP	117	117	139	139	<b>200</b>		197	217	172	172
97	MPH059	135-EGG3	IBP	117	117	139	139	198	202	<b>202</b>	<b>192</b>	170	172
97	PAS120	194-E	EPC	117	<b>119</b>	139	139		194	212	177	172	170
97	PAS120	195-E	IBP	117	117	139	139	196	194	187	212	<b>170</b>	170
97	PAS043	227-EGG3	IBP	119	117	139	139		194	212	242	<b>174</b>	174

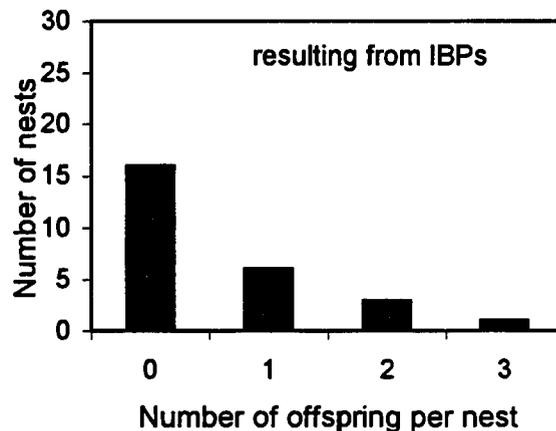
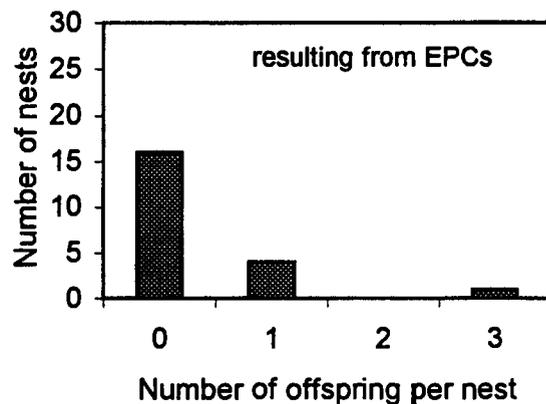
**Figure 1.** Allele frequencies at 5 microsatellite loci in Pacific Black Brant.

Allele length corresponds with sizing against internal lane standard (GS350) and locus specific “classification”. These frequencies were calculated from the 56 presumably unrelated adults. Bca4 was segregated by sex (female n=28, male n=28) due to sex linkage associated with the locus.



**Figure 2** Estimates of incidence of extra-pair copulations (EPCs) and intraspecific brood parasitism (EBP) relative to total nests.

Tables based on analysis of multilocus genotype arrays ( $n=5$  loci) for 30 nests examined during 1996 and 1997. Nests PAS015 and PAS120 each had one offspring resulting from extra-pair copulation and another offspring that resulted from intraspecific brood parasitism.



**Figure 3.** Temporal patterns of extra-pair copulations and intraspecific brood parasitism.

- A. Numbers of nests initiated throughout the colony were used to determine peak initiation in 1996 and 1997 (julian days 139 and 140, respectively). Total nests initiated were averaged between 1996 and 1997 by number of days deviating from peak initiation (left y-axis). Total numbers of nests sampled for paternity (right y-axis) are distributed throughout the 12 days of nest initiation. Days associated with 'early', 'peak', and 'late' nest initiation are defined as -6 to -3 days, -2 to 2 days, and 3-6 days, respectively.
- B. Nests containing eggs from EPCs occurred in a nonrandom distribution during peak initiation (days -2 to 2) when ca. 70% of all nests were initiated.
- C. Nests containing IBPs exhibited no temporal pattern relative to colony-wide nest initiation.

Figure 3.

