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### Evidence for Genetic Monogamy but Low Mate Retention in the North American Black Tern (*Chlidonias niger surinamensis*)

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**Abstract.**—Eleven family groups ( $n = 22$  adults, 28 chicks) of North American Black Terns (*Chlidonias niger surinamensis*) were genotyped at four polymorphic loci in a pilot study to determine the genetic mating system of this socially monogamous species. Samples were collected between 2003 and 2008 at two colony sites in Wisconsin (USA) that differed in structural complexity of the breeding habitat and nest density, and from families in which the adult males (putative fathers) varied with respect to body condition. Thus, both ecological and individual variations were explored as possible factors influencing the extent of extra-pair paternity. No mismatched alleles were detected, however, between chicks and their putative parents, suggesting that extra-pair paternity is negligible in this subspecies. Despite the small sample size, the combined probability of detecting an allelic exclusion at one or more loci was 0.998, indicating sufficient power to detect a case of extra-pair paternity if it occurred. Data obtained from recaptures of adults banded over a 10-year period (2000-2009) indicated that inter-year mate retention was low (~20%) and was observed only in pairs that remained together in consecutive years. No “divorced” mates of recaptured adults were encountered in subsequent years, suggesting that frequent mate switching is due more to the death or disappearance of the mate than to intentional dissolution of the pair bond. The results of this pilot study provide the first evidence of genetic monogamy in Black Terns and suggest that low mate fidelity is related to low breeding site fidelity, a characteristic common in birds that breed in unstable habitats. *Received 11 November 2013, accepted 19 December 2013.*

**Key words.**—Black Tern, *Chlidonias niger*, extra-pair paternity, genotyping, mate retention, mating system, microsatellite DNA.

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Despite early proclamations (e.g., Lack 1968) that most bird species are monogamous, subsequent evidence has shown that true genetic monogamy among socially monogamous birds, and passerines in particular, is relatively rare. Genetic analysis of more than 150 species of birds has revealed evidence of extra-pair paternity (EPP) in 86% of the species examined (Griffith *et al.* 2002). Despite considerable research in this area over the past 20 years, a universal explanation for EPP in birds remains elusive. Although phylogeny accounts for a considerable portion of the variation in EPP among avian lineages (Bennett and Owens 2002), finer-scale variation in the extent of EPP among related species, populations within a species, or even individuals within a population suggest a hierarchical explanation with variation determined by life history patterns, genetic considerations, or ecological, behav-

ioral, and social contexts (Griffith *et al.* 2002; Westneat and Stewart 2003).

The North American Black Tern (*Chlidonias niger surinamensis*) is a socially monogamous bird that breeds in freshwater wetlands in the United States and southern Canada (Heath *et al.* 2009). Black Terns are considered semi-colonial in breeding habit, nesting in loose aggregations in suitable wetland habitats (Shealer and Alexander 2013). Certain ecological and life-history traits of Black Terns (summarized in Heath *et al.* 2009) are correlated with high rates of EPP, including relatively high breeding densities (Westneat and Sherman 1997), structurally complex breeding habitats that may provide concealment (Sherman and Morton 1988), and a relatively synchronous breeding schedule (Stutchbury and Morton 1995; Chuang *et al.* 1999). Conversely, Black Terns possess other traits predicted to be common among mo-

nogamous species, such as the lack of obvious sexual ornaments in males (Andersson 1994) and equitable biparental care (Birkhead and Møller 1996; Møller 2000).

We conducted a pilot study to characterize the genetic mating system of Black Terns and to explore possible sources of variation in EPP, which then could be used to develop future hypothesis-driven research on this species. In particular, we genotyped family groups from two different colony sites that differed markedly in habitat complexity and nesting density, and characterized males (putative fathers) from these families according to a derived index of phenotypic quality. We incorporated data from an ongoing mark-recapture study of adults to elucidate patterns of mate retention and relate inter-year mate fidelity to the genetic mating system of this population.

## METHODS

### Study Sites and Field Procedures

Long-term population studies of Black Terns have been conducted at several colony sites in southeastern Wisconsin (USA) since 1999. Two colony sites were used in this study: Horicon Marsh (Dodge and Fond du Lac Counties, 43° 30' N, 88° 40' W) and Grassy Lake (Columbia County, 43° 25' N, 89° 10' W). The two sites differ considerably in total area and in extent and complexity of vegetative cover in the wetlands. Horicon Marsh is a very large (~13,000 ha) actively managed wetland complex dominated by cattails (*Typha* spp.), which rise vertically from the marsh to heights of 2–3 m over the growing season and obstruct visibility among nests. Grassy Lake is a much smaller (~70 ha) unmanaged and isolated wetland basin dominated by prostrate-growing vegetation (*Nymphaea odorata*, *Nuphar polysepala*) with sparse patches of emergent bulrushes (primarily *Scirpus acutus*) that offer little concealment of nests.

Nesting areas were identified first by canoe or airboat in the larger impoundments at Horicon, and individual nests were located on foot by observing the behavior of the birds after they flushed from and returned to the nest. The latitude and longitude of each nest (precision  $\pm 3$  m) was recorded when found with a GPS receiver. Nest density for each site and year was calculated by dividing the number of nests found during the early peak egg-laying period (20 May to 10 June) by the area of suitable habitat in which we worked. For Grassy Lake, suitable area was constant among years, but for Horicon Marsh we included only impoundments where birds nested in a given year and excluded open water without emergent vegetation, islands, dense stands of

cattails, and shoreline from calculation of suitable nesting area. As such, suitable habitat differed somewhat each year at Horicon due to successional changes and active water-level management and settlement decisions of the birds. For both sites, ecological density of nests was calculated by constructing polygons with the National Wetlands Inventory Wetlands Mapper (U.S. Fish and Wildlife Service 2010) and dividing the number of nests each year by the extent of suitable nesting habitat.

Adults were captured on nests during the incubation period by placing walk-in or drop-in traps over the eggs. Adults were banded with a stainless steel band and various combinations of color-bands for remote identification of individuals. Birds were weighed (to the nearest 0.5 g) with a spring scale, and various body measurements were recorded (see Shealer and Cleary 2007 for more detail). A scaled mass index (SMI) was derived separately for adults, following the procedures outlined in Peig and Green (2009), as an index of body condition of each individual at the time of capture during the incubation period. The SMI was intended to represent a relative measure of individual quality that could be compared between the sexes of each pair to test for assortative mating for this character. Of the skeletal measures recorded, head-plus-bill length was most strongly correlated with body mass (Pearson's  $r = 0.40$ ,  $n = 22$ ,  $P = 0.06$ ) and was therefore used as the single linear measure to compute the SMI, according to Equation 2 in Peig and Green (2009).

Blood samples ( $\leq 40$   $\mu$ l) were collected from each adult during trapping and from each chick within 2 days post-hatching, before they were developed enough to leave the confines of the nest site. Blood was extracted from the tarsal vein by puncture with a 27-gauge needle, collected in a capillary tube and transferred to a 1.5 ml microcentrifuge tube, labeled according to band number of the individual and containing a standard lysis buffer for storage. Adults were released immediately following processing, and chicks were returned to their nests following banding and weighing.

### Molecular Analyses and Parentage Assessment

DNA was extracted and purified from each blood sample according to standard protocols of proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation as described in Maniatis *et al.* (1982). Polymerase chain reaction (PCR) was used for sex determination and to genotype individuals. For sex determination, we used the primer set 2550F and 2718R to amplify an intron of the CHD-1 gene on the sex chromosomes (Fridolfsson and Ellegren 1999); in Black Terns the introns on the W (female) and Z (male) chromosomes differ by ~150 nucleotides and are clearly resolvable by gel electrophoresis (Shealer and Cleary 2007).

For the genotyping study, microsatellite markers at four specific loci (Table 1) were amplified by PCR. We used primer sets for two loci (Sdaat20, Sdaat27) developed for Roseate Tern (*Sterna dougallii*; Szczys *et al.* 2005) and two loci (RBG13, RBG27) developed for Red-billed Gull (*Larus novaehollandiae*; Given *et al.*

**Table 1.** Loci used to genotype adult Black Terns, number (*n*) of individuals genotyped at each locus, number of alleles and range in allele size (base pairs), optimum annealing temperature (T), observed (Ho) and expected (He) heterozygosities under Hardy-Weinberg equilibrium and exclusion power (d) for each locus.

Species	Locus	<i>n</i>	Alleles	Size (bp)	T (°C)	Ho	He	d	Source
Red-billed Gull ( <i>Larus novaehollandiae</i> )	RBG13	13	4	216-222	56	0.538	0.643	0.513	Given <i>et al.</i> 2002
	RBG27	25	13	186-216	58	0.880	0.867	0.876	
Roseate Tern ( <i>Sterna dougallii</i> )	Sdaat20	32	17	154-217	58	0.969	0.871	0.910	Szczyz <i>et al.</i> 2005
	Sdaat27	31	5	237-249	58	0.548	0.673	0.590	

2002), which were found to be polymorphic when tested on Black Terns (Szczyz *et al.* 2005; P. A. Szczyz, unpubl. data). Reactions were run in 25 µl volumes including 2 µl of template DNA and the following reagents: 1x PCR buffer, 2.0 mM MgCl<sub>2</sub> (Promega), 0.125 mM dNTP mix (Sigma), 0.25 µM of the dye-labeled forward and reverse primers developed for each locus, and 1 unit of *Taq* polymerase. Fragment analysis was conducted on PCR products with a CEQ 8000 DNA sequencer following protocols from the manufacturer. Alleles were registered as peaks and assigned a size based on comparisons to a size standard run with each sample. PCR products for each locus were run in separate wells (i.e., not multiplexed) because fragment sizes overlapped at some loci.

Genotyping results were analyzed using the Cervus software program (Field Genetics 2006). Results included allele frequencies at each locus, observed and expected heterozygosities, and probability of detecting a case of EPP at each locus. We used unrelated adults in the allele frequency analysis, including birds from several other colony sites in the region. The overall probability (D) of detecting an exclusion at one of more loci is one minus the product of one minus the probability of detection at each locus (Westneat *et al.* 1987).

Only nests from which blood samples could be obtained from both adults and at least two chicks were used in the genotyping study. Accordingly, a total of 50 birds (22 adults, 28 chicks) from 11 family groups (six families from Grassy Lake, five from Horicon Marsh) comprised the study sample. All individuals from eight families were genotyped at three loci, and three family groups were genotyped at all four loci. We considered one or more mismatched alleles between a chick and its putative parents as evidence of an extra-pair fertilization.

## RESULTS

### Nest Density

During study period (2003-2008), average nest density during the peak nesting period (20 May-10 June) differed significantly, and by at least one order of magnitude, between Horicon Marsh and Grassy Lake (Paired

*t*-test,  $t_5 = 16.2$ ,  $P < 0.001$ ). The number of nests ranged from 59-227 at Horicon Marsh (density range: 0.04-0.16 nests ha<sup>-1</sup>) and 77-105 at Grassy Lake (density range: 1.52-2.08 nests ha<sup>-1</sup>).

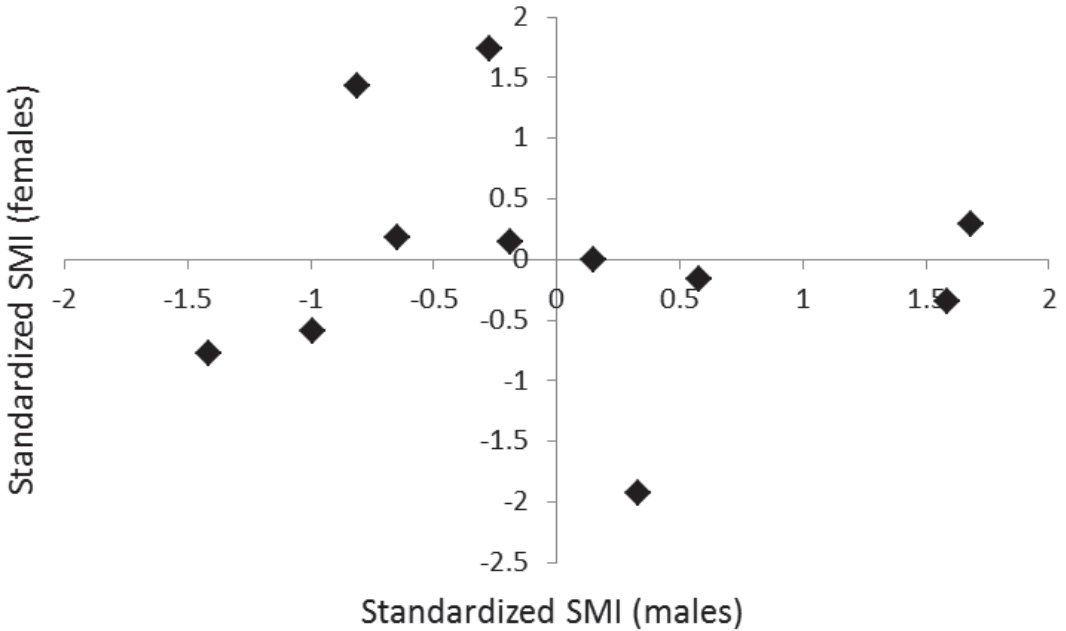
### Male Quality

Male body condition, as indexed by the SMI, was highly variable and evenly distributed, ranging from -1.5 to nearly +2.0 SD from the mean. Conversely, female body condition was less variable, with most ( $n = 8$  of 11) individuals  $\leq 1$  SD (Fig. 1). The SMI correlation between mates was inverse ( $r = -0.09$ ) but not statistically significant ( $P = 0.80$ ), providing no evidence that Black Terns paired assortatively with respect to body condition. Six of the 11 males were mated to females with a higher SMI (Fig. 1).

### Allelic Diversity and Parentage Analysis

Allelic diversity ranged from 4 to 17 alleles per locus, and two of the four microsatellite loci were highly heterozygous (Table 1). Three of the four loci deviated from Hardy-Weinberg equilibrium, with an excess of heterozygotes at Sdaat20 and lower than expected heterozygosities at RBG13 and Sdaat27.

No mismatched alleles were detected among any of the chicks and their mothers or putative fathers. The probability of detecting an exclusion at a single locus ranged from 0.54 to 0.97 (Table 1). With all four loci, the combined power of detecting a mismatch at one or more loci was 0.998. The combined exclusion power for the two loci with the highest heterozygosities (Sdaat20,



**Figure 1.** Relationship between standardized scaled mass index (SMI) of male and female Black Terns for the 11 pairs used in the genotyping study. Points to the left of the origin denote pairs in which female SMI > male SMI.

RBG27) was nearly as high ( $D = 0.989$ ). All family groups were genotyped with at least one of these highly heterozygous loci and two others; the lowest probability of detecting a mismatch for a family group genotyped at only three loci was 0.975.

### Mate Retention

Adult capture histories over a 10-year (2000-2009) period revealed low annual mate retention in this population. Of the 27 adults whose mates were known both in the year of capture and at least one year of recapture, only five (19%) of them were paired with the same bird, and all of these cases were of pairs maintained in consecutive years. Ten birds (37%) switched mates from one year to the next, and the remainder (44%) had switched mates following at least a 2-year interval between initial capture and subsequent recapture. One male was caught in three different years, each time with a different mate. No “divorced” mates of these recaptured birds were encountered in any year following the dissolution of the pair bond.

### DISCUSSION

Our genotyping results revealed no evidence of extra-pair paternity in Black Terns. Although our sample size was small, the four loci we used were sufficiently heterozygous to provide a high probability of detecting cases of EPP in these broods, if, in fact, they occurred. The results of this study suggest genetic, as well as social, monogamy in the population we studied. Because we sampled two breeding colonies that differed with respect to vegetation structure and nest density, and because some apparently low-quality males were paired with high-quality females, we were able to explore at least some known or hypothesized correlates of EPP. We had intended for this pilot study to provide a basis for future hypothesis-driven work on EPP or intraspecific brood parasitism in Black Terns, but the lack of parental exclusion in any of the 11 broods precluded further research.

The prediction that high-density breeding should promote EPP is based on hypotheses attempting to explain the prevalence of colonial nesting in birds. Because



mate-searching costs presumably are much reduced, colonial breeding may facilitate promiscuity among female birds seeking to obtain good genes from males of higher quality than their social mate. Wagner (1993, 1998) hypothesized that bird colonies may function as “hidden leks” whereby the aggregation of nests is controlled by females seeking to increase opportunities to compare potential extra-pair mates. Most parentage studies of colonial-breeding larids, however, indicate that extra-pair fertilizations are rare, if they occur. For example, there was 0% EPP in Western Gull (*L. occidentalis*; Gilbert *et al.* 1998) and Black-legged Kittiwake (*Rissa tridactyla*; Helfenstein *et al.* 2004). EPP was low in two populations of Common Tern (*S. hirundo*) where it was 0% (Griggio *et al.* 2004) and 3% (González-Solís *et al.* 2001). EPP was also low (8%) in Common Gull (*L. canus*; Bukacińska *et al.* 1998). However, see Ležalová-Piálková (2011) for an exception of 33% EPP among broods in Black-headed Gull (*L. ridibundus*). Such consistent findings argue against colonial breeding as a consequence of female-driven mate choice and suggest instead that extra-pair fertilizations may be context-dependent, influenced by social factors, genetic considerations, life history, or a combination of these and other factors.

Black Terns are among a small group of bird species considered to be semi-colonial in nesting habit. A general explanation for a semi-colonial breeding habit remains unclear, but our pilot study suggests at least that Black Terns do not form nesting aggregations to obtain extra-pair fertilizations and that local density does not seem to influence extra-pair paternity one way or the other. Semi-colonial Linnets (*Carduelis cannabina*) also exhibit a very low incidence of EPP, despite high local nest densities, intense mate guarding by males and frequent copulations within pairs (Bonlokke-Pedersen *et al.* 2002). Conversely, another semi-colonial nesting species, the Upland Sandpiper (*Bartramia longicauda*) exhibited an unexpectedly high frequency (30% of broods) of extra-pair paternity (Casey *et al.* 2011) despite the fact that this species is socially monogamous

and males provide substantial parental care. Casey *et al.* (2011) considered, but dismissed, semi-colonial nesting as an explanatory factor in promoting high EPP because local nest density was unrelated to occurrences of EPP.

The adaptive significance of extra-pair mating as it relates to genetic benefits has focused on the good genes hypothesis, that females will accept an inferior social mate but attempt to obtain extra-pair fertilizations (EPF) from males of higher quality (Birkhead and Møller 1992), and the heterozygosity hypothesis, that females seek EPFs either to increase the genetic diversity of their offspring (Williams 1975) or to maximize the genetic compatibility between themselves and the father of their offspring (Tregenza and Wedell 2000). Phenotypic cues about genetic quality may be expressed in males in the form of body size, plumage dichromatism, or sexual ornaments (Andersson 1994). Male Black Terns, however, are only slightly larger than females, do not differ from females with respect to plumage coloration, and do not possess any obvious sexual ornaments. We found no support for positive assortative pairing by body condition in our study; in fact, the relationship was inverse, with some apparently low-quality males paired with high-quality females. Under a good genes hypothesis, we might have expected to find evidence of extra-pair offspring in these broods, since mismatches in partner quality tend to promote extra-pair copulations (Petrie and Hunter 1993). Reproductive success, however, is chronically low in this population, which presented challenges in attempting to obtain a sufficient sample of entire broods to conduct this study. If biparental care is an important component in the mating system of Black Terns—and our work over the past 15 years suggests that it is—then all the males in our sample might be considered to be of higher than average quality, despite differences in body condition, since they were tending the chicks that survived long enough for us to sample them.

Despite evidence suggesting genetic monogamy during a single breeding sea-

son, mate retention among years appears to be low in this population. Recaptures of breeding adults provided no evidence that mate fidelity extended beyond two consecutive years. "Divorced" mates of recaptured adults, however, were never encountered in subsequent years, suggesting that mate switching results more from the death or disappearance of one member of the pair than from intentional dissolution of the pair bond. The former explanation is consistent with the low apparent annual survival estimates generated for Black Terns at these two colony sites (Shealer 2007). At a colony site in Oregon, Stern (1987) also found that mate retention was low (22%) in consecutive years, which he attributed to low breeding site fidelity, a characteristic common to birds that breed in unstable habitats (McNicholl 1975). Cuthbert (1985) also argued that the low frequency (25%) of mate retention she found in a population of Caspian Terns (*Hydroprogne caspia*) probably was influenced more by the inter-year stability of nest sites than by prior breeding success. Asynchronous arrival at the breeding site was the most likely explanation for divorce in Common Terns at a colony site in Germany (González-Solís *et al.* 1999). Regardless of its cause, frequent mate switching in Black Terns does not appear to be associated with genetic polygyny or driven solely by female mate choice.

Finally, although we failed to detect any evidence of EPP in *C. niger surinamensis*, we note an intriguing phenotypic difference between the North American subspecies and the Eurasian *C. niger niger*. Although *C. niger surinamensis* is not strongly dimorphic with respect to measures of body size (Shealer and Cleary 2007) or plumage, *C. niger niger* can exhibit considerable dichromatism, with females having contrastingly grayer feathering on the throat and belly compared to the uniform black feathering of males (Olsen and Larson 1995). This plumage difference is obvious enough in most pairs to enable sex determination in the field, which usually is not possible for *C. niger surinamensis*. Whether this plumage difference is a sexually-selected trait is unknown, but it suggests that the criteria for mate choice may differ between the two subspecies.

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