

GENETIC PARENTAGE AND LOCAL POPULATION STRUCTURE IN THE SOCIALLY MONOGAMOUS UPLAND SANDPIPER

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Abstract. For a single lineage of birds, the diversity of mating systems and parental care among shorebirds (Charadrii) is high, which has made them an important group for investigations of the evolution of social mating systems. From 2003 to 2007, we studied a population of the Upland Sandpiper (*Bartramia longicauda*) at Konza Prairie Biological Station in northeastern Kansas. Our objectives were to determine the genetic mating system of this socially monogamous shorebird and to determine whether spatial patterns of philopatry and nest placement might affect opportunities for extra-pair mating. We used six microsatellite markers to estimate rates of extra-pair paternity in 58 family groups (107 parents, 184 offspring). We found that 30% of broods contained extra-pair offspring, representing 15% of chicks, the highest rate of extra-pair paternity ever reported in a socially monogamous shorebird. High rates of extra-pair paternity were not due to the degree of relatedness between partners in mated pairs, and they did not result in greater diversity among broods with extra-pair young. We used a spatial genetic-autocorrelation analysis and found evidence for relatedness among females nesting <1 km apart. The Upland Sandpiper might have a high rate of extra-pair paternity because related females nest synchronously and in close proximity, but the probability of extra-pair young was not related to nest density or distance to nearest nest. Female-biased natal philopatry is unusual among birds but is consistent with the mate-defense mating system of the Upland Sandpiper.

Key words: *Bartramia longicauda*, dispersal, extra-pair paternity, genetic structure, mating systems, natal philopatry, shorebirds, Upland Sandpiper.

Parentesco Genético y Estructura Poblacional en *Bartramia longicauda*, una Especie Socialmente Monógama

Resumen. Para un único linaje, la diversidad de sistemas de apareamiento y cuidado parental entre las aves costeras (Charadrii) es alto, lo que ha hecho de éstos un grupo importante para la investigación de la evolución de los sistemas sociales de apareamiento. Desde 2003 a 2007, estudiamos una población de *Bartramia longicauda* en la Estación Biológica Konza Prairie en el noreste de Kansas. Nuestros objetivos fueron determinar el sistema de apareamiento genético de esta ave costera socialmente monógama y determinar si los patrones espaciales de filopatría y posición de los nidos pueden afectar las oportunidades de apareamientos fuera de la pareja. Utilizamos seis marcadores microsatelitales para estimar la tasa de paternidad fuera de la pareja en 58 grupos familiares (107 padres, 184 crías). Encontramos que el 30% de las nidadas contenían crías engendradas por fuera de la pareja, representando un 15% de los polluelos, la mayor tasa de paternidad por fuera de la pareja que se conoce para un ave costera socialmente monógama. Las altas tasas de paternidad por fuera de la pareja no se debieron al grado de parentesco entre los miembros de una pareja, y no resultaron en una mayor diversidad en las nidadas con polluelos engendrados por fuera de la pareja. Usamos un análisis de autocorrelación especial genética y encontramos evidencia de parentesco entre hembras que anidaban separadas por < 1 km. Esta especie puede tener una alta tasa de paternidad por fuera de la pareja porque las hembras emparentadas anidan de forma sincrónica y próximas entre sí, pero la probabilidad de tener polluelos engendrados por fuera de la pareja no se correlacionó con la densidad de nidos o la distancia al nido más próximo. La filopatría sesgada hacia las hembras es poco común entre las aves, pero es consistente con el sistema de apareamiento de defensa de pareja de *B. longicauda*.

INTRODUCTION

Molecular genetic techniques have advanced the understanding of avian biology in several areas, including the ecology of dispersal and settlement patterns (Double et al. 2005), evolution of mating systems (Thomas et al. 2007), and the

development of conservation strategies (Manel et al. 2003, Temple et al. 2006). Many of these advances have been due to the revelation of unexpected phenomena, such as fine-scale genetic structure among nesting birds and high rates of extra-pair paternity (EPP). The genetic structure of bird populations has been studied over large geographic areas, but, until recently,

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few studies have examined fine-scale genetic structure. Recent studies have examined kin associations among lek-mating birds (Höglund et al. 1999, Shorey et al. 2000, Bouzat and Johnson 2004) as well as genetic structure with respect to dispersal behavior (Double et al. 2005, Temple et al. 2006, Woxvold et al. 2006) and patterns of nest placement (Foerster et al. 2006, Andersson and Waldeck 2007). Additionally, genetic studies have revealed that EPP is common in birds, which has led to significant research into the variation and ecological correlates of EPP rates within and among species (Westneat et al. 1990, Birkhead and Møller 1992, Petrie and Kempenaers 1998). Previous studies of EPP have focused largely on songbirds (61%, $n = 131$ species) but levels of EPP may be different in other orders of birds (Griffith et al. 2002).

In the shorebirds (order Charadriiformes, suborder Charadrii) mating systems and patterns of parental care are diverse (Reynolds and Székely 1997, Székely et al. 2006, Thomas et al. 2007). Most species breeding in the North Temperate Zone and Arctic lay a clutch of 3 or 4 eggs and rear precocial young that feed themselves (MacLean 1972, Sandercock 1997). An invariant clutch size may constrain reproductive strategies because annual fecundity can be increased only through multiple nesting attempts (Ligon 1993). In species with precocial young, one parent may be capable of incubating or raising the brood alone, which may give the second parent the option of deserting to remate and produce another clutch (Székely et al. 1996). Costs of parental care lead to a sexual conflict over which parent should care for the offspring and the duration of care. As a result, shorebirds' social mating systems are diverse and include monogamy, polygyny, polyandry, and promiscuity. Despite this great variability, genetic investigations of breeding systems have been completed for relatively few shorebirds (16 of 155 species, Piersma et al. 1997).

We studied the population genetics and mating system of the Upland Sandpiper (*Bartramia longicauda*), a socially monogamous shorebird that breeds in the grasslands of North America and winters in the pampas of South America. Unlike many shorebirds, the Upland Sandpiper is completely terrestrial and is rarely found along coasts. It is somewhat unusual in that males are not territorial and in the breeding season home ranges in grasslands are large and overlapping (>200 ha, Mong 2005). Indeed, mated pairs have been found to nest in loose colonies (Buss and Hawkins 1939, Bowen 1976, Bowen and Kruse 1993). Despite overlapping territories, males have a mate-defense mating system and guard their mate before the female lays (Houston and Bowen 2001). Females lay a 4-egg clutch, both sexes incubate the eggs jointly, and males are responsible for attending the mobile young after hatching, as females depart from the study area within 0–5 days of hatching (Houston and Bowen 2001; B. K. Sandercock, unpubl. data). Females may reneest if they lose a clutch, but pairs do not produce more than one brood per year.

Our study objectives were to determine the rate of EPP and ecological correlates of its variation in a population of

Upland Sandpipers in northeast Kansas. We tested four hypotheses: (1) We expected to find low rates of EPP because males contribute extensively to parental care during incubation and brood rearing, and the risk of abandonment or reduced paternal care may increase if males have less certainty of paternity (Griffith et al. 2002). (2) As we did not find the rate of EPP to be low, we tested the hypothesis that EPP arises to decrease the genetic relatedness of offspring when parents are closely related. Socially monogamous shorebirds tend to have low rates of EPP, but genetic similarity between partners in mated pairs is correlated with higher rates of EPP in at least three species (Blomqvist et al. 2002a), suggesting that EPP might function to decrease potential inbreeding when parents are closely related. We tested whether genetic similarity affects Upland Sandpipers' decisions in mating and predicted that the probability of EPP should be positively related to the genetic relatedness of mated pairs (Amos et al. 2001). Furthermore, if females select genetically dissimilar males for extra-pair copulations, the heterozygosity of extra-pair offspring should be higher (Smith et al. 2005). (3) Density of the breeding population has also been proposed as an ecological correlate of EPP (Birkhead and Møller 1992, Hayes et al. 2006), where opportunities to pursue extra-pair copulations should be greater when individuals are nesting in close proximity. If density affects EPP, we expected a positive relationship between increased spatial proximity of nests and the rate of EPP. (4) The fine-scale genetic structure of breeding populations of birds has rarely been examined (Temple et al. 2006). We tested whether the semi-colonial spatial structure of nesting Upland Sandpipers had an effect on the species' genetic mating system.

METHODS

FIELD METHODS

We sampled mated pairs of Upland Sandpipers at Konza Prairie Biological Station, Manhattan, Kansas, over 5 years (2003–2007). Konza Prairie is a 3487-ha preserve of tallgrass prairie, but our research was confined to ~1500 ha at higher elevations in the southern third of the preserve. We captured adult sandpipers at night in early spring with the use of spotlights and long-handled nets. We attached a radio transmitter (PD-2, Holohil Systems Ltd., ON, Canada) to each sandpiper and tracked each radio-marked bird until we located its nest (Mong and Sandercock 2007). We then used mist nets to capture the mate of each radio-marked bird at the nest during incubation. At first capture, we banded each sandpiper with a numbered metal band, a unique combination of colored plastic bands, and collected a 200- μ L blood sample from the brachial wing vein.

We estimated the date of hatching at each nest by egg floatation and captured chicks at the nest on the day of hatching (Liebezeit et al. 2007). We collected blood (~20 μ L) from all chicks by clipping the toenail. To increase our sample of family groups, we sampled one clutch of four eggs that failed during late

TABLE 1. Optimization of six microsatellite markers for genotyping of Upland Sandpipers. Metrics reported include heterozygosity expected under assumptions of Hardy–Weinberg equilibrium (H_E), observed heterozygosity (H_O), number of observed alleles, and optimum annealing temperature (T_a).

Species	Locus	H_E	H_O	Observed alleles	T_a (°C)	Source
Eurasian Oystercatcher (<i>Haematopus ostralegus</i>)	4A11	0.860	0.861	14	60	Van Treuren et al. 1999
	49F6	0.733	0.704	11	55	
Dunlin (<i>Calidris alpina</i>)	Calp2	0.665	0.639	8	53	Wennerberg and Bensch 2001
Ruff (<i>Philomachus pugnax</i>)	Ruff1	0.619	0.763 ^a	8	52	Thuman et al. 2002
	Ruff6	0.900	0.628 ^a	21	52	
	Ruff8	0.842	0.762 ^a	12	48	

^aSignificant departure from Hardy–Weinberg equilibrium.

incubation when embryo development was nearly complete. In 2007, we also temporarily removed three complete clutches and replaced the eggs with clay dummy eggs, painted to resemble real eggs. Eggs were placed in an incubator (model D-2362-N Hova-Bator; G.Q.F. Mfg. Co., Savannah, GA) and monitored until hatching. Eggs were automatically turned throughout incubation. At hatching, we measured the chicks, collected a blood sample, and returned the chicks to the original nest. In all cases, the attending parent accepted the brood. The three artificially incubated clutches represented 5% of the 57 nests and 5% of all hatched eggs in the study ($n = 12$ of 222 eggs). Blood samples were stored in the dark in Queen's lysis buffer at 5 °C until DNA extractions were completed (Seutin et al. 1991).

GENETIC ANALYSES

Using the DNeasy kit (Qiagen, Inc., Valencia, CA), we extracted DNA from the blood of 131 adults and 177 chicks, and from samples of brain tissue from four unhatched embryos. Nest predation was high, and adults were rarely successful in multiple years. One adult male was included in the analysis in two different years, but we accepted a low degree of pseudoreplication to make full use of our sample of family groups. We sexed all individuals by molecular markers with the primers P2/P8 or 2550F/2718R (Griffiths et al. 1998, Fridolfsson and Ellegren 1999, Casey et al. 2009). Using fluorescently labeled primers (Schuelke 2000), we amplified microsatellite markers by the polymerase chain reaction in an Eppendorf Mastercycler ep gradient thermal cycler (Eppendorf, Westbury, NY) and ran them on an ABI Prism 3730 Genetic Analyzer. We visualized allele sizes with GeneMarker software version 1.6 (SoftGenetics, LLC, State College, PA). We genotyped each bird at six microsatellite loci developed for three other species of shorebirds, 4A11, 49F6 (Eurasian Oystercatcher, *Haematopus ostralegus*; Van Treuren et al. 1999), Calp2 (Dunlin, *Calidris alpina*; Wennerberg and Bensch 2001), Ruff1, Ruff6, and Ruff8 (Ruff, *Philomachus pugnax*; Thuman et al. 2002; Table 1). We ran homozygous individuals twice to minimize genotyping errors caused by potential allelic dropout.

PARENTAGE ANALYSIS

Prior to exclusion analyses, we checked for null alleles and deviations from Hardy–Weinberg equilibrium with the program Micro-Checker (Van Oosterhout et al. 2004). Ruff8 is a sex-linked locus on the Z chromosome, so we used males to calculate levels of expected and observed heterozygosity (Thuman et al. 2002). We also used half genotypes of females for the parentage analysis (Van Treuren 1998). The observed level of heterozygosity at each locus deviated from Hardy–Weinberg equilibrium at three of the loci, with Ruff1 having an excess of heterozygotes and Ruff6 and Ruff8 having a deficit. We verified the accuracy of the loci that were out of Hardy–Weinberg equilibrium by revisualizing all mismatches between known pairs of parent and offspring (Hoffman and Amos 2005).

At each nest, we assessed parentage by comparing the genotypes of the offspring and of the adults attending the nest. We calculated the combined exclusion power of our six loci with the program Cervus; it was $P > 0.996$ if one parent was known (Kalinowski et al. 2007). A deficiency of heterozygotes can lead to false mismatches between parents and offspring if a shared allele is lost because of allelic dropout (Bonin et al. 2004). Since heterozygotes were deficient for Ruff6 and Ruff8, we used a conservative approach and considered offspring to be the result of extra-pair mating when they mismatched at two or more loci with their putative father or mother. In every case where exclusion was based on two mismatches, at least one of the two loci was in Hardy–Weinberg equilibrium. Additionally, a complete lack of mismatches between putative mothers and offspring led us to conclude that despite deviations from Hardy–Weinberg equilibrium, our assignments of paternity were reliable (Marshall et al. 1998).

GENETIC SIMILARITY

For a measure of genetic similarity between partners in mated pairs and different individuals nesting near each other, we estimated pairwise relatedness with the program Relatedness (version 5.0.8; Queller and Goodnight 1989). Estimates of relatedness depend on accurate knowledge of alleles' background

frequencies, so we excluded Ruff8 from this analysis. We used two methods to confirm that our markers were sufficient to provide accurate estimates of relatedness between individuals. First, we used the rarefaction analysis of Girman et al. (1997), in which we selected a locus randomly and calculated the relatedness of two randomly selected individuals. We then randomly selected a second locus and recalculated relatedness with both loci and continued this process until all loci were selected. We then expressed the difference in relatedness between consecutive samplings as a function of the total number of loci used. Second, we used *t*-tests to compare the mean pairwise relatedness estimates to the expected values for birds of known relationships, including first-order relatives, half-siblings, and mated pairs.

We calculated standardized individual heterozygosity (hereafter, "heterozygosity") for each individual as the proportion of heterozygous loci per average observed heterozygosity for all microsatellite loci genotyped. By use of a standardized measure, we eliminated bias toward individuals that were genotyped at all loci over those birds where amplification failed at one or more loci (Coltman et al. 1999). We excluded the sex-linked locus Ruff8 from all calculations of heterozygosity.

SPATIAL PROXIMITY

To examine the effects of density of nests on the probability of extra-pair young, we estimated this density by nearest-neighbor distances between nests, the number of neighboring nests within 0.5 km, and the number of nests within 1 km. Our estimates of this density should be viewed as indices because Upland Sandpipers are secretive during incubation and not all nests were discovered (Smith et al. 2009).

GENETIC STRUCTURE AMONG NESTING BIRDS

We pooled data from our 5-year study (2003–2007) to analyze, using the program Spagedi (Hardy and Vekemans 2002), the relationship between pairwise relatedness and geographic distance among nests in the same year for adult males and females. In this analysis, we excluded known re-nesting attempts and used locations of first nesting attempts. To increase our sample size, we genotyped and included an additional 23 adult sandpipers that attended unsuccessful nests in 2006. We calculated the genetic similarity and nearest-neighbor distances for each year's cohort, and then combined the results for all years. The mean distance between a nest and its nearest neighbor was 347 m (± 25 m, range 2–6029 m), so we categorized nest distances into five classes: 0.0–0.5 km, 0.5–1.0 km, 1.0–2.0 km, 2.0–3.0 km, and >3 km. We computed average values of relatedness for each distance interval, jackknifing over the five microsatellite loci to obtain standard errors for each estimate of relatedness. We tested for a trend in relatedness vs. pairwise distance between nests and used permutation tests to determine the significance of the relatedness estimate in

each distance class. The permutation procedure is analogous to performing a Mantel test between matrices of genetic and geographic distances (Hardy and Vekemans 2002).

STATISTICAL ANALYSES

We examined three correlates of EPP: degree of relatedness within mated pairs, heterozygosity of offspring, and an index of density of the breeding population. We used logistic regression to calculate estimates of the relationship of mates' relatedness and three measures of population density on the probability of extra-pair fertilization. We used paired *t*-tests to determine the significance of differences in heterozygosity between within-pair and extra-pair young. We analyzed data for broods in which all four chicks were genotyped and for all broods combined (1–4 chicks genotyped) separately. Results for both datasets were identical (Casey 2008), so we report results for all broods combined. We ran statistical analyses with procedures of the programs JMP (version 4.0.4., SAS Institute, Cary, NC), SAS (version 8.0.2., SAS Institute, Cary, N.C.), and R (version 2.9.2, R Foundation for Statistical Computing, Vienna, Austria). We considered results of statistical tests significant at $P \leq 0.05$ and report estimates \pm SE.

RESULTS

MICROSATELLITES

The six microsatellite markers showed high levels of heterozygosity (range in H_o : 0.628–0.861), and allelic diversity ranged from 8 to 21 alleles per locus (Table 1). There were no mismatches in loci between offspring and putative mothers, except at one unusual nest where we trapped three incubating birds. Of 177 chicks, the number of offspring that did not match the putative father was 18% for 1 locus, 4% for 2 loci, and 11% for >2 loci. We considered offspring to be the result of extra-pair mating if they did not match the putative father at >2 loci. In nearly all cases where there was a single mismatch, the mismatch was at the locus Ruff6. Between extra-pair offspring and their excluded parents the mean number of mismatched loci was 3.15 (± 0.21 , $n = 27$) for the six loci tested.

SOCIAL MATING SYSTEM

We examined parentage in 57 family groups monitored over 5 years (108 adults and 181 chicks). The 181 chicks genotyped were 82% of the 222 potential young produced by these adults. Samples were missed because of partial predation of clutches, inviable eggs that failed to hatch, and chicks evading capture if we misjudged the expected date of hatching and attempted to capture the brood after it departed the nest. In total, we genotyped both parents in 50 family groups, only the female parent in one family group, and only the attending male in six family groups. Across all 5 years of the study, nearly all pairs formed socially monogamous pair bonds and incubated the clutch jointly (97%, $n = 86$). We found three cases where

social pairings differed from a single male with a single female. In 2004, one nest was attended by three birds, and we obtained genetic samples from one male and one female; the third bird was not sampled. The female did not match the offspring at four of six loci, and the third bird may have been the true genetic mother. Additionally, in 2006 and 2007 there were two nests attended by two females. Elsewhere, we have shown that with the P2/P8 primers male Upland Sandpipers can be missexed as females (Casey et al. 2009), but in these cases we are confident of our sex assignments because we sexed all sandpipers with two independent sets of primers. In the case of the female–female pairs, all four birds were heterozygous with both sets of primers. Although we found a few instances of unusual associations, social monogamy was the predominant mating system in the population we studied.

GENETIC MATING SYSTEM

Across all 5 years of the study, 30% of 56 broods contained at least one offspring sired by an extra-pair male, and 15% of 177 chicks were sired by an extra-pair male (Table 2). For neither chicks ($\chi^2_4 = 3.5$, $P = 0.47$) nor broods ($\chi^2_4 = 1.2$, $P = 0.88$) did the frequency of extra-pair fertilizations vary by year. One of 51 broods was attended by an unrelated female, representing 2% of 164 chicks, and the only nest with an extra-pair female was the nest attended by three birds in 2004. Fourteen broods had mixed paternity and contained both within-pair and extra-pair offspring. Ten broods with mixed paternity, where all four chicks were captured, contained an average of 1.4 extra-pair chicks per brood, with six nests containing one extra-pair chick and four nests containing two extra-pair chicks. Sample sizes varied because complete information was not available for all nests.

GENETIC SIMILARITY

Rarefaction analyses based on bootstrap procedures indicated that estimates of relatedness were unbiased when the number of loci was ≥ 4 (Fig. 1). Pairwise estimates of relatedness for first-order relatives identified through parentage assignment averaged 0.48 ± 0.01 ($n = 155$), which was significantly different

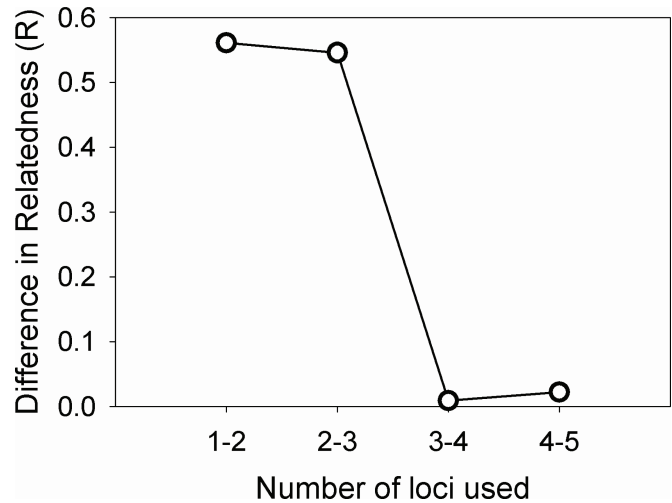


FIGURE 1. Relationship between the number of loci used and the difference in consecutive estimates of relatedness.

from but close to the expected value of $r = 0.5$ (one-sample t -test; $t_{154} = 2.0$, $P = 0.047$). Half-siblings identified by parentage assignment had a lower relatedness value of $r = 0.31 \pm 0.03$ ($n = 14$), which was not significantly different from the expected value of $r = 0.25$ ($t_{13} = 2.0$, $P = 0.07$). Mated pairs were the least closely related group with $r = 0.002 \pm 0.03$ ($n = 50$), which was not different from the expected r value of zero for a fully outbred population ($t_{49} = 0.07$, $P = 0.95$).

CORRELATES OF EXTRA-PAIR PATERNITY

We found no indication that females were choosing extra-pair mates on the basis of relatedness to their social partner. There was no relationship between the degree of relatedness of mated pairs and the presence of extra-pair offspring (logistic regression, $\chi^2_1 = 0.4$, $P = 0.52$). Per brood, the heterozygosity of extra-pair young tended to be greater (1.04 ± 0.08) than that of within-pair young (0.96 ± 0.08), but the difference ($+0.07 \pm 0.06$, $n = 14$ broods) was not significant (paired t -test, $t = 0.67$, $P = 0.51$). Furthermore, the heterozygosity of offspring from broods that contained no extra-pair young (0.98 ± 0.02) did not differ from that of offspring from broods of mixed paternity (0.99 ± 0.07 , $t = -0.05$, $P = 0.48$). There was no relationship between the presence of extra-pair young and the number of neighbors nesting within 0.5 km (logistic regression, $\chi^2_1 = 0.8$, $P = 0.36$, Fig. 2A) or 1 km ($\chi^2_1 = 0.3$, $P = 0.57$, Fig. 2B). Similarly, the presence of extra-pair young was not related to the distance to the nearest nest ($\chi^2_1 = 0.46$, $P = 0.49$, Fig. 2C).

GENETIC POPULATION STRUCTURE

When we tested for deviations from an expected random distribution of genotypes, we found evidence that breeding females settled nonrandomly. Females nesting nearest each other were significantly more related than expected by chance in distance classes <0.5 km ($P = 0.02$) and 0.5–1 km ($P = 0.04$, Fig. 3A).

TABLE 2. Annual variation in rates of extra-pair paternity in Upland Sandpipers at Konza Prairie Biological Station, Kansas, 2003–2007.

Year	Chicks		Broods	
	Percent	<i>n</i>	Percent	<i>n</i>
2003	18.8	48	35.3	17
2004	13.9	36	27.2	11
2005	3.8	26	14.2	7
2006	21.0	38	33.3	12
2007	13.8	29	33.3	9
Total	15.2	177	30.4	56

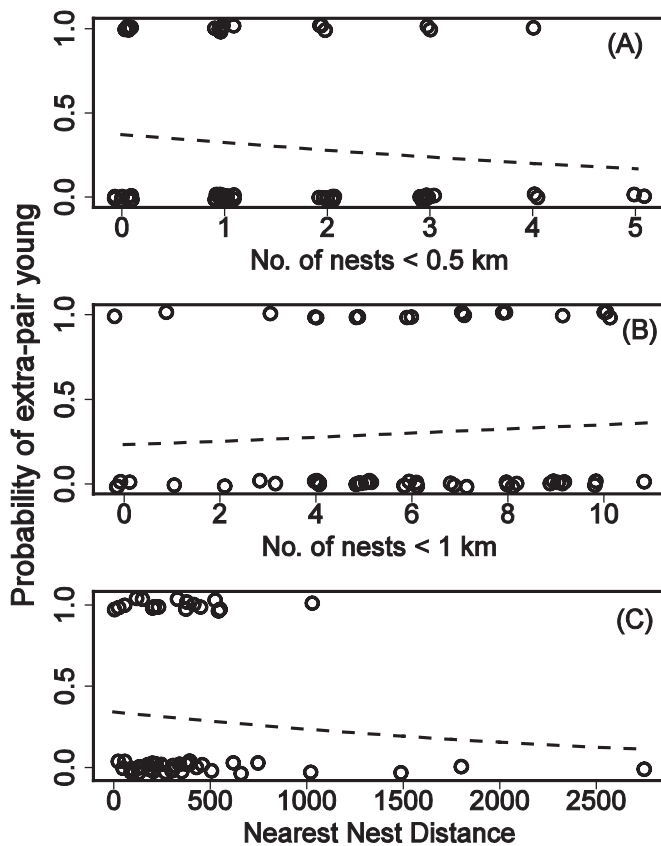


FIGURE 2. Relationships between the presence of extra-pair young and the number of adjacent neighbors within 0.5 km (A), the number of adjacent neighbors within 1 km (B), and the distance to nearest neighboring nests (C).

Relatedness values of r from 0.05 to 0.09 for females nesting <1 km apart are intermediate between the r values of 0.031 and 0.125 expected for second and first cousins, respectively. In contrast, females at intermediate distances of 2–3 km were less related than expected ($P = 0.049$). Males did not deviate significantly from a random distribution of genotypes (Fig. 3B).

DISCUSSION

Rates of EPP we found in Upland Sandpipers breeding in northeast Kansas (30% of broods) were at the extreme upper end of the range previously reported for shorebirds with socially monogamous or sequentially polyandrous mating systems (EPP was found in <10% of broods in 9 of 12 species, Table 3). High rates of EPP are unusual among shorebirds that breed as mated pairs but have been reported in the Common Sandpiper (*Actitis hypoleucos*; 19%), Southern Lapwing (*Vanellus chilensis*; 19%), and Red Phalarope (*Phalaropus fulicarius*; 33%). In fact, the EPP rate we found in the Upland Sandpiper is intermediate between rates of multiple paternity in shorebirds with simultaneous polyandry where females have pair bonds with multiple males (18–21% in two species)

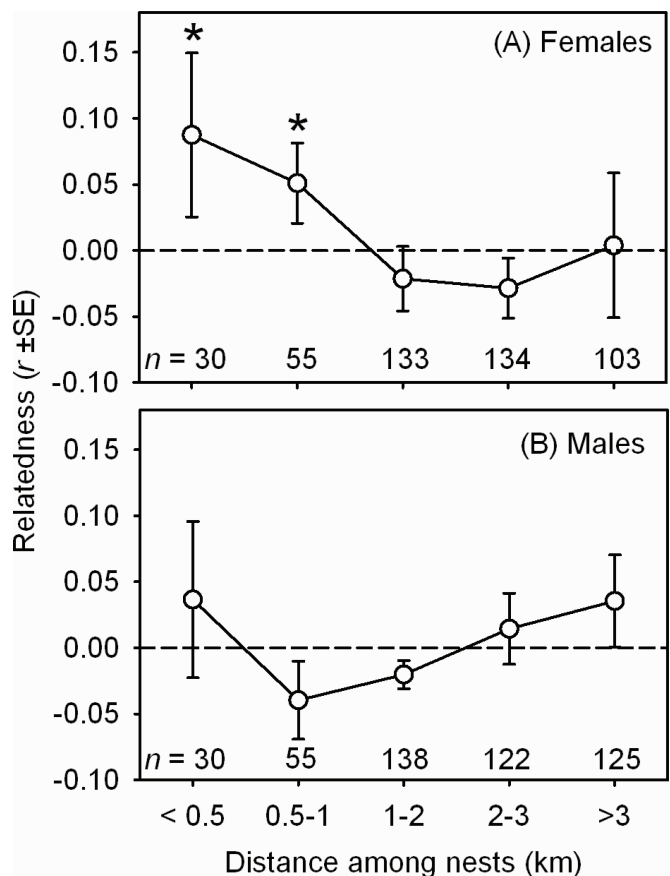


FIGURE 3. Average pairwise relatedness among female (A) and male (B) Upland Sandpipers in relation to the distance among nests in the same year. For each distance class, the observed and expected mean relatedness were compared by a permutation test. Asterisks indicate observations that differ significantly from the expected value ($P < 0.05$). Error bars show standard errors obtained by jackknifing over loci. Numbers above the x axis indicate the number of pairwise comparisons for each distance class.

and in lek-mating shorebirds that mate promiscuously without pair bonds (>40% in two species, Table 3).

One possible explanation for the high rate of EPP we observed is that Upland Sandpipers are nonterritorial and have large overlapping home ranges (>200 ha, Mong 2005). Observations of individually marked Upland Sandpipers between arrival and the onset of nesting indicate a mate-defense mating system. In this social system, unmated males use the “wolf-whistle” call as a sexual display, mated males guard their mates to assure paternity, and copulations are only rarely observed (Houston and Bowen 2001; B. K. Sandercock, unpubl. data). Opportunities for extra-pair mating for shorebirds that defend mates may differ from those for shorebirds that instead defend territories used for foraging and breeding, if the two strategies differ in rates of encounter with receptive females or opportunities for extra-pair mating. For instance, the Red Phalarope is a nonterritorial species with mate-defense polyandry, and its rate of EPP is also high (Dale et al. 1999).

TABLE 3. Rates of multiple paternity of shorebirds in relation to social mating system.

Mating system and species	% Extra-pair paternity		Parental care ^a		Source
	Young (<i>n</i>)	Broods (<i>n</i>)	Eggs	Young	
Monogamous					
Upland Sandpiper <i>Bartramia longicauda</i>	15.2 (177)	30.4 (56)	MF	M	This study
Southern Lapwing <i>Vanellus chilensis</i>	9.8 (41)	18.8 (16)	MF	MF	Saracura et al. 2008
Common Sandpiper <i>Actitis hypoleucos</i>	15.7 (83)	18.5 (27)	MF	M(F)	Mee et al. 2004
Western Sandpiper <i>Calidris mauri</i>	5.0 (98)	8.0 (40)	MF	M(F)	Blomqvist et al. 2002b
Kentish Plover <i>Charadrius alexandrinus</i>	3.9 (229)	7.9 (89)	MF	M(F)	Küpper et al. 2004
	0.6 (170)	1.5 (65)			Blomqvist et al. 2002a
Semipalmated Plover <i>Charadrius semipalmatus</i>	4.7 (85)	4.0 (24)	MF	MF	Zharikov and Nol 2000
Eurasian Oystercatcher <i>Haematopus ostralegus</i>	1.5 (65)	3.8 (26)	MF	MF	Heg et al. 1993
Purple Sandpiper <i>Calidris maritima</i>	1.2 (82)	3.7 (27)	MF	M	Pierce and Lifjeld 1998
Ringed Plover <i>Charadrius hiaticula</i>	0 (57)	0 (21)	MF	MF	Wallander et al. 2001
Polyandrous: sequential					
Red Phalarope <i>Phalaropus fulicarius</i>	8.6 (70)	33.3 (18)	M	M	Dale et al. 1999
Eurasian Dotterel <i>Charadrius morinellus</i>	4.5 (44)	9.1 (22)	M	M	Owens et al. 1995
Red-necked Phalarope <i>Phalaropus lobatus</i>	1.7 (226)	6.0 (63)	M	M	Schamel et al. 2004
Wilson's Phalarope <i>Phalaropus tricolor</i>	0.0 (43)	0.0 (15)	M	M	Delehanty et al. 1998
Polyandrous: simultaneous					
Spotted Sandpiper <i>Actitis macularius</i>	10.8 (111)	20.6 (34)	M	M	Oring et al. 1992
Wattled Jacana <i>Jacana jacana</i>	7.5 (235)	17.9 (74)	M	M	Emlen et al. 1998
Promiscuous/lekking					
Ruff <i>Philomachus pugnax</i>	—	59.0 (34)	F	F	Lank et al. 2002
	—	44.1 (34)			Thuman and Griffith 2005
Buff-breasted Sandpiper <i>Tryngites subruficollis</i>	—	40.4 (47)	F	F	Lanctot et al. 1997

^aM = male only, F = female only, MF = biparental, M(F) = biparental but predominantly male.

A mate-defense mating system without territoriality may also have implications for the Upland Sandpiper's observed population structure. In a spatial analysis of genetic relatedness, we found that females, but not males, nesting <1 km apart were more closely related than expected by chance, and at levels of relatedness intermediate between those of first and second cousins. The spatial pattern of relatedness can be explained only by joint settlement of female siblings or by female-biased natal philopatry, both of which are unusual among birds. Females are usually the dispersive sex because birds tend to have resource-defense mating systems where males defend resources to attract females (Greenwood 1980, Clarke et al. 1997). The exceptions to male-biased natal dispersal tend to be species with mate-defense mating systems, such as the Upland Sandpiper, or waterfowl and other birds that pair at nonbreeding sites (Robertson and Cooke 1999).

Semi-colonial nesting of the Upland Sandpiper has been reported previously at several other sites (Buss and Hawkins 1939, Bowen 1976, Bowen and Kruse 1993), and our discovery of local genetic structure suggests that patterns of nest aggregation are caused by females preferentially settling near relatives. The coefficients of relatedness we found among adjacent females were relatively low ($r < 0.1$) but could facilitate kin selection. Observers disturbing nests and broods leads to joint mobbing by flying sandpipers giving a distinctive vocalization ("tattler call" of Houston and Bowen 2001). Thus

settlement near kin could lead to inclusive fitness if related females from adjacent nests warn of approaching predators.

Semi-colonial nesting could also be a partial explanation for the Upland Sandpiper's rates of EPP being higher than those of other species of socially monogamous shorebirds. Increased population densities can reduce the costs of extra-pair mating and facilitate higher frequencies of extra-pair copulation (Westneat and Sherman 1997). Reduced costs of locating extra-pair mates may benefit females with clustered nesting territories, and the benefits may lead to colony formation as an adaptive advantage (Wagner 1993). However, we found no evidence to support the prediction that EPP should be correlated with population density. The number of adjacent neighbors and nearest-neighbor distance were not related to the presence of extra-pair offspring. However, we were unable to control for missing nests because for secretive, ground-nesting shorebirds nest detectability is likely to be <1 (Smith et al. 2009). Therefore, we cannot rule out the true density of the breeding population as a factor affecting EPP in the Upland Sandpiper.

Male-female pairing was the most common social pair bond we observed in the Upland Sandpiper. It is possible that we underestimated the rates of other social arrangements because our field protocol was to discontinue trapping after capturing the mate of the radio-marked bird. The low frequency of nests attended by female-female pairs or groups of three birds (2% of nests) is comparable to the frequency of supranormal

clutches reported in past studies of the Upland Sandpiper. In south-central North Dakota, Bowen and Kruse (1993) found that 1.8% of 342 nests contained more than the modal clutch of 4 eggs (range 5–7) and hypothesized that these clutches could be the result of joint egg laying by multiple females. We observed one nest with five eggs, but the clutch was depredated before we could collect samples for parentage analyses. Molecular methods have revealed unusual female behaviors in other species of shorebirds, including “quasi-parasitism,” in which extra-pair females contribute to a clutch after copulating with a male, resulting in a brood fathered by a single male but with mixed maternity (Küpper et al. 2004). Rates of quasi-parasitism range from 3.1% in the Kentish Plover (*Charadrius alexandrinus*) to 13.3% in the Common Sandpiper but are not associated with supranormal clutches (Blomqvist et al. 2002a, Grønstøl et al. 2006). Joint attendance of a nest by more than two birds is rare among shorebirds but does occur in some populations of the Southern Lapwing (Saracura et al. 2008) and American Oystercatcher (*Haematopus palliatus*, Lauro et al. 1992). To our knowledge, female–female pairs have not been previously reported for shorebirds but are known from some seabird populations where adult sex ratios are female-biased (Hunt and Hunt 1977, Nisbet and Hatch 1999).

We found no evidence to support the hypothesis that female Upland Sandpipers choose extra-pair mates on the basis of genetic similarity, which has been the most widely supported of the genetic hypotheses for shorebirds (Blomqvist et al. 2002a) and songbirds (Kempnaers et al. 1999, Foerster et al. 2003). We found that the relatedness of mated pairs with and without extra-pair young did not differ, and neither did the heterozygosity of within-pair and extra-pair young. Because we were unable to assign paternity to extra-pair fathers, however, the possibility remains that extra-pair males could have been less related to the females than were within-pair males (Griffith et al. 2002). Additionally, coefficients of relatedness based on our neutral microsatellite markers may not reflect differences between males at important functional and regulatory genes, and they may not reveal subtle differences in genetic diversity and relatedness, so females could be choosing males on the basis of other genetic factors (Reed and Frankham 2001).

In conclusion, we have reported the first estimates of EPP for the Upland Sandpiper. Our results are intriguing because this species has among the highest rates of EPP of any socially monogamous shorebird studied to date, and our conclusions are robust because our samples were larger than in most previous studies of EPP in shorebirds and were based on microsatellite loci. We rejected genetic similarity between mated pairs and increases in heterozygosity as explanations for this result. Instead, high rates of EPP may be related to unusual patterns of settlement by philopatric females in a species with a mate-defense mating system. Fine-scale genetic structure among nesting females suggests either natal philopatry of females or females settling preferentially near kin. Nesting in colonies

may provide opportunities for females to gain extra-pair copulations, regardless of relatedness. Our study is one of the first applications of microsatellites in a genetic study of a shorebird’s mating system. Previous work has been primarily based on multi-locus markers, which have been criticized for estimation of genetic similarity (Griffith and Montgomerie 2003, but see Blomqvist et al. 2003). Development of improved field techniques is necessary for extra-pair mates to be identified and the survival and recruitment of young after their departure from the nest to be determined. Answers to those questions could provide insights into the adaptive advantages of EPP for the Upland Sandpiper. In the future, application of analyses of fine-scale spatial genetic autocorrelation and highly variable microsatellite markers will offer new insights into the evolution of mating systems in shorebirds and other birds.

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LITERATURE CITED

- AMOS, W., W. J. WORTHINGTON, K. FULLARD, T. M. BURG, J. P. CROXALL, D. BLOCH, AND T. COULSON. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B* 268:2021–2027.
- ANDERSSON, M., AND P. WALDECK. 2007. Host–parasite kinship in a female-philopatric bird population: evidence from a relatedness trend analysis. *Molecular Ecology* 16:2797–2806.
- BIRKHEAD, T. R., AND A. P. MØLLER. 1992. *Sperm competition in birds: evolutionary causes and consequences*. Academic Press, London.
- BLOMQVIST, D., M. ANDERSSON, C. KUPPER, I. C. CUTHILL, J. KIS, R. B. LANCTOT, B. K. SANDERCOCK, T. SZÉKELY, J. WALLANDER, AND B. KEMPENAERS. 2002a. Genetic similarity between mates and extra-pair parentage in three species of shorebirds. *Nature* 419:613–615.
- BLOMQVIST, D., B. KEMPENAERS, R. B. LANCTOT, AND B. K. SANDERCOCK. 2002b. Genetic parentage and mate guarding in the arctic-breeding Western Sandpiper. *Auk* 119:228–233.
- BLOMQVIST, D., M. ANDERSSON, C. KUPPER, I. C. CUTHILL, J. KIS, R. B. LANCTOT, B. K. SANDERCOCK, T. SZÉKELY, J. WALLANDER, AND B. KEMPENAERS. 2003. Why do birds engage in extra-pair copulation? *Nature* 422:833–834.
- BONIN, A., E. BELLEMAIN, P. B. EIDSEEN, F. POMPANON, C. BROCHMANN, AND P. TABERLET. 2004. How to track and assess genotyping errors

- in population genetics studies. *Molecular Ecology* 13:3261–3273.
- BOUZAT, J. L., AND K. JOHNSON. 2004. Genetic structure among closely spaced leks in a peripheral population of Lesser Prairie Chickens. *Molecular Ecology* 13:499–505.
- BOWEN, D. E. JR. 1976. Coloniality, reproductive success, and habitat interactions in Upland Sandpipers (*Bartramia longicauda*). Ph.D. dissertation, Kansas State University, Manhattan, KS.
- BOWEN, B. S., AND A. D. KRUSE. 1993. Effects of grazing on nesting by Upland Sandpipers in south-central North Dakota. *Journal of Wildlife Management* 57:291–301.
- BUSS, I. O., AND A. S. HAWKINS. 1939. The Upland Plover at Faville Grove, Wisconsin. *Wilson Bulletin* 51:202–220.
- CASEY, A. E. 2008. Fine-scale genetic structure and extra-pair parentage in the socially monogamous Upland Sandpiper. M.Sc. thesis, Kansas State University, Manhattan, KS.
- CASEY, A. E., K. L. JONES, B. K. SANDERCOCK, AND S. M. WISELY. 2009. Heteroduplex molecules cause sexing errors in a standard molecular protocol for avian sexing. *Molecular Ecology Resources* 9:61–65.
- CLARKE, A. L., B. E. SÆTHER, AND E. RØSKAFT. 1997. Sex biases in avian dispersal: a reappraisal. *Oikos* 79:429–438.
- COLTMAN, D. W., J. G. PILKINGTON, J. A. SMITH, AND J. M. PEMBERTON. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259–1267.
- DALE, J., R. MONTGOMERIE, D. MICHAUD, AND P. BOAG. 1999. Frequency and timing of extrapair fertilisation in the polyandrous Red Phalarope (*Phalaropus fulicarius*). *Behavioral Ecology and Sociobiology* 46:50–56.
- DELEHANTY, D. J., R. C. FLEISCHER, M. A. COLWELL, AND L. W. ORING. 1998. Sex-role reversal and the absence of extra-pair fertilization in Wilson's Phalaropes. *Animal Behaviour* 55:995–1002.
- DOUBLE, M. C., R. PEAKALL, N. R. BECK, AND A. COCKBURN. 2005. Dispersal, philopatry, and infidelity: dissecting local genetic structure in Superb Fairy-wrens (*Malurus cyaneus*). *Evolution* 59:625–635.
- EMLEN, S. T., P. H. WREGGE, AND M. S. WEBSTER. 1998. Cuckoldry as a cost of polyandry in the sex-role-reversed Wattled Jacana, *Jacana jacana*. *Proceedings of the Royal Society of London B* 265:2359–2364.
- FOERSTER, K., K. DELHEY, A. JOHNSEN, J. T. LIJFELD, AND B. KEMPENAERS. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* 425:714–717.
- FOERSTER, K., M. VALCU, A. JOHNSON, AND B. KEMPENAERS. 2006. A spatial genetic structure and effects of relatedness on mate choice in a wild bird population. *Molecular Ecology* 15:4555–4567.
- FRIDOLFSSON, A.-K., AND H. ELLEGREN. 1999. A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology* 30:116–121.
- GIRMAN, D. J., M. G. L. MILLS, E. GEFFEN, AND R. K. WAYNE. 1997. A molecular genetic analysis of social structure, dispersal and interpack relationships of the African wild dog (*Lycaon pictus*). *Behavioral Ecology and Sociobiology* 40:187–198.
- GREENWOOD, P. J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. *Animal Behaviour* 28:1140–1162.
- GRIFFITH, S. C., I. P. F. OWENS, AND K. A. THUMAN. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11:2195–2212.
- GRIFFITH, S. C., AND R. MONTGOMERIE. 2003. Why do birds engage in extra-pair copulation? *Nature* 422:833.
- GRIFFITHS, R., M. C. DOUBLE, K. ORR, AND R. J. G. DAWSON. 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075.
- GRØNSTØL, G., D. BLOMQUIST, AND R. H. WAGNER. 2006. The importance of genetic evidence for identifying intra-specific brood parasitism. *Journal of Avian Biology* 37:197–199.
- HARDY, O. J., AND X. VEKEMANS. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2:618–620.
- HAYES, M. A., H. B. BRITTEN, AND J. A. BARZEN. 2006. Extra-pair fertilizations in Sandhill Cranes revealed using microsatellite DNA markers. *Condor* 108:970–976.
- HEG, D., B. J. ENS, T. BURKE, L. JENKINS, AND J. P. KRUIT. 1993. Why does the typically monogamous oystercatcher (*Haematopus ostralegus*) engage in extra-pair copulations? *Behaviour* 126:247–289.
- HOFFMAN, J. I., AND W. AMOS. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology* 14:599–612.
- HÖGLUND, J., R. V. ALATALO, A. LUNDBERG, P. T. RINTAMAKI, AND J. LINDELL. 1999. Microsatellite markers reveal the potential for kin selection on Black Grouse leks. *Proceedings of the Royal Society of London B* 266:813–816.
- HOUSTON, C. S., AND D. E. BOWEN JR. 2001. Upland Sandpiper (*Bartramia longicauda*), no. 580. In A. Poole and F. Gill [EDS.], *The birds of North America*. Birds of North America, Inc., Philadelphia.
- HUNT, G. L. JR., AND M. W. HUNT. 1977. Female–female pairing in Western Gulls (*Larus occidentalis*) in southern California. *Science* 196:1466–1467.
- KALINOWSKI, S. T., M. L. TAPER, AND T. C. MARSHALL. 2007. Revisiting how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16:1099–1106.
- KEMPENAERS, B., B. CONGDON, P. BOAG, AND R. J. ROBERTSON. 1999. Extrapair paternity and egg hatchability in Tree Swallows: evidence for the genetic compatibility hypothesis? *Behavioral Ecology* 10:304–311.
- KÜPPER, C., J. KIS, A. KOSZTOLÁNYI, T. SZÉKELY, I. C. CUTHILL, AND D. BLOMQUIST. 2004. Genetic mating system and timing of extra-pair fertilizations in the Kentish Plover. *Behavioural Ecology and Sociobiology* 57:32–39.
- LANCOT, R. B., K. T. SCRIBNER, B. KEMPENAERS, AND P. J. WEATHERHEAD. 1997. Lekking without a paradox in the Buff-breasted Sandpiper. *American Naturalist* 149:1051–1070.
- LANK, D. B., C. M. SMITH, O. HANOTTE, A. OHTONEN, S. BAILEY, AND T. BURKE. 2002. High frequency of polyandry in a lek mating system. *Behavioral Ecology* 13:209–215.
- LAURO, B., E. NOL, AND M. VICARI. 1992. Nesting density and communal breeding in American Oystercatchers. *Condor* 94:286–289.
- LIEBEZEIT, J. R., P. A. SMITH, R. B. LANCOT, H. SCHEKKERMAN, I. TULP, S. J. KENDALL, D. M. TRACY, R. J. RODRIGUES, H. MELTOFFE, J. A. ROBINSON, C. GRATTO-TREVOR, B. J. MCCAFFERY, J. MORSE, AND S. W. ZACK. 2007. Assessing the development of shorebird eggs using the flotation method: species-specific and generalized regression models. *Condor* 109:32–47.
- LIGON, J. D. 1993. The role of phylogenetic history in the evolution of contemporary avian mating and parental care systems. *Current Ornithology* 10:1–46.
- MACLEAN, G. L. 1972. Clutch size and evolution in the Charadrii. *Auk* 81:299–324.
- MANEL, S., M. K. SCHWARTZ, G. LUIKART, AND P. TABERLET. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* 18:189–197.
- MARSHALL, T. C., J. SLATE, L. E. B. KRUK, AND J. M. PEMBERTON. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639–655.
- MEE, A., D. P. WHITFIELD, D. B. A. THOMPSON, AND T. BURKE. 2004. Extrapair paternity in the Common Sandpiper, *Actitis hypoleucos*, revealed by DNA fingerprinting. *Animal Behaviour* 67:333–342.

- MONG, T. W. 2005. Using radio-telemetry to determine range and resource requirements of Upland Sandpipers at an experimentally managed prairie landscape. M.Sc. thesis, Kansas State University, Manhattan, KS.
- MONG, T. W., AND B. K. SANDERCOCK. 2007. Optimizing radio retention and minimizing radio impacts in a field study of Upland Sandpipers. *Journal of Wildlife Management* 71:971–980.
- NISBET, I. C. T., AND J. J. HATCH. 1999. Consequences of a female-biased sex-ratio in a socially monogamous bird: female–female pairs in the Roseate Tern *Sterna dougallii*. *Ibis* 141:307–320.
- ORING, L. W., R. C. FLEISCHER, J. M. REED, AND K. E. MARSDEN. 1992. Cuckoldry through stored sperm in the sequentially polyandrous Spotted Sandpiper. *Nature* 359:631–633.
- OWENS, I. P. F., A. DIXON, T. BURKE, AND D. B. A. THOMPSON. 1995. Strategic paternity assurance in the sex-role reversed Eurasian Dotterel (*Charadrius morinellus*)—behavioral and genetic evidence. *Behavioral Ecology* 6:14–21.
- PETRIE, M., AND B. KEMPENAERS. 1998. Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology and Evolution* 13:52–58.
- PIERCE, E. P., AND J. T. LIFJELD. 1998. High paternity without paternity assurance behavior in the Purple Sandpiper, a species with high paternal investment. *Auk* 115:602–612.
- PIERSMA, T., P. WIERSMA, AND J. VAN GILS. 1997. The many unknowns about plovers and sandpipers of the world: introduction to a wealth of research opportunities highly relevant for shorebird conservation. *Wader Study Group Bulletin* 82:23–33.
- QUELLER, D. C., AND K. F. GOODNIGHT. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- REED, D. H., AND R. FRANKHAM. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* 55:1095–1103.
- REYNOLDS, J. D., AND T. SZÉKELY. 1997. The evolution of parental care in shorebirds: life histories, ecology, and sexual selection. *Behavioral Ecology* 8:126–134.
- ROBERTSON, G. J., AND F. COOKE. 1999. Winter philopatry in migratory waterfowl. *Auk* 116:20–34.
- SANDERCOCK, B. K. 1997. Incubation capacity and clutch size determination in two calidrine sandpipers: a test of the four-egg threshold. *Oecologia* 110:50–59.
- SARACURA, V., R. H. MACEDO, AND D. BLOMQUIST. 2008. Genetic parentage and variable social structure in breeding Southern Lapwings. *Condor* 110:554–558.
- SCHAMEL, D., D. M. TRACY, D. B. LANK, AND D. F. WESTNEAT. 2004. Mate guarding, copulation strategies and paternity in the sex-role reversed, socially polyandrous Red-necked Phalarope *Phalaropus lobatus*. *Behavioral Ecology and Sociobiology* 57:110–118.
- SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18:233–234.
- SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69:82–90.
- SHOREY, L., S. PIERTNEY, J. STONE, AND J. HÖGLUND. 2000. Fine-scale genetic structuring on *Manacus manacus* leks. *Nature* 408:352–353.
- SMITH, S. B., M. S. WEBSTER, AND R. T. HOLMES. 2005. The heterozygosity theory of extra-pair mate choice in birds: a test and a cautionary note. *Journal of Avian Biology* 36:146–154.
- SMITH, P. A., J. BART, R. B. LANCTOT, B. J. MCCAFFERY, AND S. BROWN. 2009. Probability of detection of nests and implications for survey design. *Condor* 111:414–423.
- SZÉKELY, T., G. H. THOMAS, AND I. C. CUTHILL. 2006. Sexual conflict, ecology, and breeding systems in shorebirds. *Bioscience* 56:887–887.
- SZÉKELY, T., J. N. WEBB, A. I. HOUSTON, AND J. M. MCNAMARA. 1996. An evolutionary approach to offspring desertion in birds. *Current Ornithology* 13:271–330.
- TEMPLE, H. J., J. I. HOFFMAN, AND W. AMOS. 2006. Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the White-breasted Thrasher, *Ramphocinclus brachyurus*. *Molecular Ecology* 15:3449–3458.
- THOMAS, G. H., T. SZÉKELY, AND J. D. REYNOLDS. 2007. Sexual conflict and the evolution of breeding systems in shorebirds. *Advances in the Study of Behavior* 37:279–342.
- THUMAN, K. A., F. WIDEMO, AND S. B. PIERTNEY. 2002. Characterization of polymorphic microsatellite DNA markers in the Ruff (*Philomachus pugnax*). *Molecular Ecology Notes* 2:276–277.
- THUMAN, K. A., AND S. C. GRIFFITH. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour* 69:765–770.
- VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLS, AND P. SHIPLEY. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535–538.
- VAN TREUREN, R. 1998. Estimating null allele frequencies at a microsatellite locus in the oystercatcher (*Haematopus ostralegus*). *Molecular Ecology* 7:1413–1417.
- VAN TREUREN, R., R. BIJLSMA, J. M. TINBERGEN, D. HEG, AND L. VAN DE ZANDE. 1999. Genetic analysis of the population structure of socially organized oystercatchers (*Haematopus ostralegus*) using microsatellites. *Molecular Ecology* 8:181–187.
- WAGNER, R. H. 1993. The pursuit of extra-pair copulations by female birds—a new hypothesis of colony formation. *Journal of Theoretical Biology* 163:333–346.
- WALLANDER, J., D. BLOMQUIST, AND J. T. LIFJELD. 2001. Genetic and social monogamy—does it occur without mate guarding in the Ringed Plover? *Ethology* 107:561–572.
- WENNERBERG, L., AND S. BENSCH. 2001. Geographic variation in Dunlin *Calidris alpina* as revealed by morphology, mtDNA and microsatellites. Ph.D. dissertation, Lund University, Lund, Sweden.
- WESTNEAT, D. F., AND P. W. SHERMAN. 1997. Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology* 41:205–215.
- WESTNEAT, D. F., P. W. SHERMAN, AND M. L. MORTON. 1990. The ecology and evolution of extra-pair copulations in birds. *Current Ornithology* 7:331–369.
- WOXVOLD, I. A., G. J. ADCOCK, AND R. A. MULDER. 2006. Fine-scale genetic structure and dispersal in cooperatively breeding Aposlebirds. *Molecular Ecology* 15:3139–3146.
- ZHARIKOV, Y., AND E. NOL. 2000. Copulation behavior, mate guarding, and paternity in the Semipalmated Plover. *Condor* 102:231–235.