



EXTREME LEVELS OF MULTIPLE MATING CHARACTERIZE THE MATING SYSTEM OF THE SALTMARSH SPARROW (*AMMODRAMUS CAUDACUTUS*)

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ABSTRACT.—We studied mating patterns in Saltmarsh Sparrows (*Ammodramus caudacutus*), a species with no territorial defense, no paternal care, and no pair bonds. Fifty-seven of 60 broods with at least 2 sampled chicks (and all broods that were completely sampled) resulted from multiple mating by females. About one-third of the broods had a different father for each chick, but the most common pattern, found in 36 of 60 nests, was multiple sires, with at least 1 male having sired 2 or more chicks. The level of multiple paternity in Saltmarsh Sparrows may be the highest documented in any bird. Levels of multiple mating increased with population density but not with nesting synchrony. We were able to assign paternity to particular males for about half the 206 chicks that were genotyped. Males sired chicks as far as 1.4 km from their original capture site, and some males sired chicks in multiple nests, separated by up to 0.5 km. We also document a case of 2 females laying eggs in the same nest. Received 30 June 2009, accepted 8 October 2009.

Key words: *Ammodramus caudacutus*, mating system, paternity, polyandry, promiscuity.

Niveles Extremos de Apareamientos Múltiples Caracterizan el Sistema de Apareamiento de *Ammodramus caudacutus*

RESUMEN.—Estudiamos los patrones de apareamiento en *Ammodramus caudacutus*, una especie que no presenta defensa territorial, ni cuidado parental ni formación de parejas duraderas. Cincuenta y siete de 60 nidadas para las cuales se muestrearon al menos dos polluelos (y todas las nidadas que fueron muestreadas completamente) fueron el resultado de apareamientos múltiples por parte de las hembras. Aproximadamente un tercio de las nidadas tuvieron un padre diferente para cada polluelo, pero el patrón más común (encontrado en 36 de 60 nidos) fue de progenitores múltiples, con dos o más polluelos engendrados por al menos un macho. El nivel de paternidad múltiple en esta especie puede ser el más alto documentado para cualquier especie de ave. Los niveles de apareamiento múltiple aumentaron con la densidad poblacional, pero no con la sincronía de anidación. Fuimos capaces de asignar la paternidad a un macho en particular para más o menos la mitad de los 206 polluelos para los cuales establecimos el genotipo. Los machos engendraron polluelos a distancias de hasta 1.4 km de su lugar de captura original y algunos machos engendraron polluelos en varios nidos, separados por hasta 0.5 km. También documentamos un caso de dos hembras que pusieron sus huevos en el mismo nido.

GENETIC PATERNITY STUDIES have revealed a diversity of avian mating systems undreamed of when mating systems were inferred from field observations of social behavior and copulations. Most paternity studies have been conducted on socially monogamous species, and the best-known outcome of this genetic work has been the insight that many species that were previously considered monogamous engage in out-of-pair copulations. From Lack's (1968) assertion that 90% of bird species were monogamous, the field has evolved to the point where we now understand that 90% of those supposedly monogamous species actually engage in some level of multiple mating (Griffith et al. 2002), making the more precise term "socially monogamous" a necessary part of the vocabulary.

Mating systems and division of parental care appear to be evolutionarily labile, responding to environmental pressures such as the abundance and distribution of resources for feeding young (Emlen and Oring 1977). Another distinct outcome of genetic paternity studies has been to highlight species whose mating systems stretch monogamy, polygyny, and other simplistic categories (Lack 1968, Emlen and Oring 1977) to the breaking point: social breeders in which 76% of young are sired by out-of-group males (Mulder et al. 1994); "monogamous" birds in which more than half the young arise from extrapair copulations (Barber et al. 1996); polyandrous female sandpipers that store sperm from one mate to fertilize eggs later laid in another male's nest (Oring et al. 1992); and the extremely odd Greater Vasa Parrot (*Coracopsis vasa*), in

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which almost every nest has multiple paternity, females sing, and multiple males provision the females but do not feed the young (Ekstrom et al. 2007).

Beyond the general diversity of mating systems and the extreme examples cited above, the broadest general point has been that mating with multiple individuals of the opposite sex is, across all bird species, more the rule than the exception. Although males can accrue obvious fitness benefits from mating with multiple females, corresponding benefits to females from mating with multiple males are less obvious, because female fecundity is not obviously limited by access to mating opportunities. Explaining the prevalence of (and variation in) female multiple mating has been one of the most persistent challenges in the field. Explanations for multiple mating behavior by females have largely been sought within the framework of extrapair copulations by monogamously mated females (Griffith 2007). Comparative studies have found that differences in the rate of extrapair fertilizations among species can be tied to a broad range of factors, some historical, some population genetic, some physiological or morphological (see table 1 in Westneat and Stewart 2003), but firm conclusions are sometimes elusive because explanatory factors are often correlated with one another. Those synthesizing work on the evolution of female multiple mating have often specifically excluded species without a monogamous pair bond (Westneat et al. 1990, Hasselquist and Sherman 2001, Griffith et al. 2002, Westneat and Stewart 2003). Other than simplification and the preponderance of studies on socially monogamous species, however, there is no clear reason to exclude lekking and other nonmonogamous species. Further investigation of mating patterns in nonmonogamous species, in fact, seems likely to help broaden our perspective on multiple mating by female birds.

Saltmarsh Sparrows (*Ammodramus caudacutus*) breed exclusively in tidal marshes from Maine to North Carolina and are unusual among songbirds in that they do not defend territories, lack conspicuous breeding displays, do not form pair bonds, and exhibit female-only parental care (Woolfenden 1956, Greenlaw and Rising 1994). Food is apparently abundant during nesting, which allows females to raise their young without help from males (Post and Greenlaw 1982, Gjerdrum et al. 2008b). The species occurs at high breeding densities (Gjerdrum et al. 2008a), and nesting is often synchronized following the simultaneous flooding of many nests during high spring tides (Gjerdrum et al. 2005, Shriver et al. 2007). Both high density and high nesting synchrony in bird populations have been suggested as predictors of multiple paternity (Møller and Birkhead 1993, Stutchbury and Morton 1995, Griffith 2007).

The Saltmarsh Sparrow's mating system has been described as "promiscuous" (Woolfenden 1956, Post and Greenlaw 1982, Greenlaw and Rising 1994), and its origins as "mysterious" (Greenlaw and Rising 1994), but published descriptions of mating behaviors are sparse. A color-banded female has been observed mating with multiple males at different times (Greenlaw and Rising 1994), and males have been observed copulating with multiple females (Woolfenden 1956). Greenlaw and Rising (1994: 11) stated that receptive females perform "Copulation Invitation display away from the nest . . . when a male approaches." Greenlaw and Rising (1994: 11) also indicated that males "often force copulations on unreceptive females," but we have been unable to find a published

description of a forced copulation. Woolfenden (1956: 61) asserted that copulation "occurs most frequently in the course of, or immediately following, a fight between several males" and wrote that he "found it difficult to distinguish fighting males from a copulating pair." Woolfenden described one observed copulation in detail but did not report any forced copulations. Given this unusual combination of characteristics, Saltmarsh Sparrows would appear to be well suited for detailed investigations of avian mating behavior, especially female choice, but direct quantification of paternity patterns in the species are lacking. In the present study, therefore, our primary objective is to provide detailed information on the incidence and pattern of multiple paternity within broods to better quantify female mating behavior. We also test whether the degree of multiple paternity is related to the local abundance of males or to nesting synchrony. Finally, we provide information on the degree to which males sire chicks in multiple nests from the same, and from different, females.

METHODS

Field methods and site descriptions.—We conducted this work concurrently with field work for studies of Saltmarsh Sparrow habitat use and demography (e.g., Gjerdrum 2005, 2008a; consult those studies for detailed field methods and a map of the study sites). Samples were deliberately spread out across nine marshes along 67 km of coastline in Connecticut to ensure that our results would be broadly applicable. Study marshes ranged in size from 26 to 289 ha, with population sizes varying accordingly. Sightings of banded birds indicate that Saltmarsh Sparrows move around within marshes frequently, and between marshes at least occasionally. In 2003, we sampled at Barn Island Wildlife Management Area, Stonington ($n = 3$ broods); Black Hall River Marsh, Old Lyme (2); East River Marsh, Guilford (4); Great Island Wildlife Management Area, Old Lyme (14); Hammock River Marsh, Clinton (2); and Hammonasset State Park, Madison (8). In 2004, we sampled at Back River Marsh, Old Saybrook (2); East (6) and West (5) River Marshes, Guilford; Hammock River Marsh, Clinton (2); Hammonasset State Park, Madison (5); and Patagansett River Marsh, East Lyme (9).

Within each marsh, we established multiple 1-ha study plots, and data from 30 plots across the nine marshes were included in the present study. Adults were captured using mist nets in each study plot, sexed by presence or absence of a cloacal protuberance or brood patch, and color-banded. We used a standardized protocol to sample each plot five times over the course of a breeding season, and the total number of Saltmarsh Sparrows caught in each plot was determined (Gjerdrum et al. 2008a). Nests were located by systematic plot searches, and some that were found opportunistically outside plot boundaries were included in the sample. For nests found during egg laying or when hatching date was known, nest initiation was estimated by counting days backwards to the start of egg laying (Gjerdrum et al. 2005, 2008a). For other nests, we back-counted from the chick's age, which was estimated on the basis of developmental benchmarks (Woolfenden 1956, Greenlaw and Rising 1994) and comparisons with known-age chicks. If a banded female was seen carrying food to a nest or identified as it flushed from or entered a nest, we considered it to be the mother. If a nest was attended by an unbanded female, we attempted to capture the female as it left the nest or arrived with

food. Field observations were not sufficient to associate females with every nest, and at 2 nests, 2 females were identified as possible mothers. All field assignments of maternity were later checked using genetic data. One nest, found on 24 June 2003, contained 8 eggs. Average clutch size (\pm SD) of Saltmarsh Sparrows in a New York study was 3.9 ± 0.63 (range: 2–5, $n = 176$; Greenlaw and Rising 1994), and clutch sizes in our population did not differ substantially from that, so we suspected that more than 1 female laid in the 8-egg nest. We were not able to associate a banded female with that nest in the field, but we were able to obtain blood samples from 6 of 7 chicks that survived to near fledging age.

Bleeding, sample preservation, DNA purification, and genotyping.—Adults were bled at the time of initial capture, and chicks at age 7–10 days. We drew 30–60 μ L of blood by brachial venipuncture, transferred it to 750 μ L of lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5% SDS, pH 8.0), and kept samples at 4°C until extraction. When chicks died during nest-flooding events, we attempted to obtain blood or other tissue to use in genotyping. We also took embryonic tissue from abandoned unhatched eggs at several nests, but none of the embryonic samples could be successfully genotyped. We purified DNA using a standard phenol-chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989). DNA was reconstituted in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.0) at 20 ng μ L⁻¹. We used 12 polymorphic microsatellite loci to genotype each individual, including 9 loci developed specifically for our study (Anonymous 2008, Hill et al. 2008), plus GF12 (Petren 1998), Esc μ 06 (Hanotte et al. 1994), and As μ 15 (Bulgin et al. 2003). The number of alleles per locus in this population (mean \pm SD) was 15.7 ± 7.6 (range: 8–36), and observed heterozygosity was 0.79 ± 0.05 (range: 0.69–0.87). Theoretical non-exclusion probability for all loci combined was 0.00025 (first parent) or 0.0000019 (second parent). Heterozygosity and exclusion probabilities were calculated with CERVUS, version 3.03 (Kalinowski et al. 2007). Polymerase chain reaction (PCR) was carried out in 8- μ L volumes with dye-labeled primers (for details of buffer and thermal cycling conditions, see Hill and Post 2005, Hill et al. 2008). The PCR products were electrophoresed through an ABI 310 Genetic Analyzer, and we used GENESCAN, version 3.1, and GENOTYPER, version 2.5 (Applied Biosystems, Foster City, California), to analyze output and bin alleles. We visually checked all allele calls. Because locus Aca21 showed null alleles at a frequency of ~ 0.12 (Hill et al. 2008), we converted all apparent homozygotes at that locus to artificial heterozygotes by assigning them a “dummy” allele before analysis. This approach is conservative and allowed us to use some of the information available at that locus for paternity assignments while avoiding false exclusion of true parents (Jones and Ardren 2003).

Maternity check.—We entered genotype data from all genotyped chicks and all adult females from all marshes, along with population allelic frequencies from all adults genotyped, into CERVUS, specifying neither the mother–chick associations determined in the field nor the marsh where each female and chick were banded. We then used CERVUS to assign mothers to each chick and used output from that analysis to correct maternity assignments before attempting paternity analyses.

Paternity assignment.—To determine how many males fathered chicks in each brood, we adopted two complementary approaches. The program COLONY, version 1.2 (Wang 2004), uses

chick genotypes, maternal genotypes if available, and population allelic frequencies to estimate which chicks within each brood are full siblings and which are half siblings. COLONY thus estimates the most likely number of fathers responsible for each brood. CERVUS, on the other hand, uses all the above data plus genotypes of potential fathers and attempts to assign a father to each chick. Both approaches have weaknesses: COLONY assigns each chick to a sibship even when the chick or family is poorly genotyped—“most likely” in this case does not carry a confidence value to indicate how likely (Wang 2004). CERVUS is more “cautious,” assigning paternity to chicks only if it can do so with a predetermined level of confidence based on a simulation procedure (Marshall et al. 1998), but CERVUS is useful only if a chick’s father has, in fact, been caught, bled, and genotyped. CERVUS is thus mute on chicks whose father was not sampled. To test the robustness of the two analytical strategies for assigning paternity, we compared output from the two programs for all cases where comparisons were possible. If CERVUS assigned paternity to ≥ 2 chicks in a brood, we could examine COLONY output to determine whether COLONY agreed or disagreed with CERVUS as to whether the chicks were full or half siblings.

We used the following parameters in our CERVUS analysis of paternity. (1) Proportion of loci typed = 0.895 (based on our data). (2) Proportion of loci mistyped = 0.03 (estimated by CERVUS from mother–offspring conflicts in our data as 0.02; to be conservative, we used a higher figure because earlier correction of some errors may have reduced the proportion of errors that we could detect by comparing mother and offspring genotypes). (3) Number of candidate fathers = 46 (mean number of males sampled per marsh; number sampled ranged from a low of 8 in the smallest marsh to a high of 142 in the largest; varying this number across the full range of plausible values produced only small differences in the results). Proportion of candidate fathers sampled = 0.5. We have no way of knowing for certain what proportion of potential fathers was sampled, but banding data suggested that we captured a substantial proportion of the birds present in our survey plots, though far from all of them. Trials that use values between 0.3 and 0.7 for this parameter tended to converge on the same assignments, which suggests that our analysis is robust to uncertainty in this parameter as well. We used a 95% confidence criterion—when we use the words “assigned by CERVUS,” we mean assigned with 95% confidence.

For 7 females, we had blood samples from chicks in 2 successive nests. In addition to analyzing paternity with COLONY within each of those 14 broods, we also lumped all chicks from each female, essentially asking COLONY to treat each female’s 2 nests as 1 superbrood and to look for full sibships across nests as well as within them.

Quantifying promiscuity.—Different metrics have been used to calculate the degree to which females mate multiply, but some of these, such as the percentage of broods with multiple paternity, conflate the level of multiple mating with the likelihood of detection: at a given frequency of multiple mating, one is more likely to detect multiple paternity in larger broods. We calculated a brood-size-independent index of promiscuity by calculating, for each brood of ≥ 2 chicks, Simpson’s index of diversity (Magurran 2004) and then computing an average Simpson’s index across all nests. For each brood, we also determined the number of males

that fathered chicks. This latter number is the minimum number of fathers that contributed to the clutch, because many families were only partially sampled as a result of egg or chick losses early in the nesting cycle.

To determine whether the degree of multiple paternity was related to the number of males in the vicinity of a nest, we randomly selected 1 nest plot⁻¹ ($n = 30$ plots; mean (\pm SE) number of males plot⁻¹ = 14.9 ± 1.4 ; range: 3–33) and used linear regression to test how well our promiscuity index and the minimum number of fathers were explained by the number of males captured in a plot during standardized banding (see Gjerdrum et al. 2008a). To test whether promiscuity was related to nesting synchrony, we used nest initiation dates for all nests found in our study marshes to determine the proportion of nests in a given year that were initiated on each day of the nesting season ($n = 83$ nests in 2003, $n = 52$ in 2004; samples not limited to those broods for which paternity was assessed). For this analysis, we assumed that the daily risk of nest failure is correlated across marshes, on the grounds (1) that high spring tides cause most nest failure (Gjerdrum et al. 2005, Greenberg et al. 2006), synchronizing nesting behavior (Shriver et al. 2007); and (2) that the tidal cycle is linked to the lunar cycle and, thus, is synchronized across study sites. Each nest was assigned a “synchrony” index, equal to the proportion of nests initiated on the same day, and linear regression was used to test whether the index explained variation in the level of promiscuity.

RESULTS

Birds included in analysis: Genotyping results.—We genotyped 206 chicks from 62 nests. Our sample included 10 two-chick broods, 23 three-chick broods, 20 four-chick broods, and 7 five-chick broods. Two nests had only 1 genotyped chick each but were included because the mother also had another brood in the study, so 204 chicks from 60 multiple-chick nests were available for assessing multiple paternity within a nest. In the above totals, we count 1 nest that had 6 genotyped chicks as 2 broods of 3 chicks each; see below. All chicks were sampled in 26 nests; because of egg or chick losses early in the nesting cycle, 25 broods were only partially sampled; the remaining 9 broods were discovered too late in the nesting cycle for us to know whether or not there had been losses prior to our blood sampling. For the 51 nests for which we had a complete egg count, an average (\pm SD) of 0.65 ± 0.74 young per nest went unsampled.

Of the 62 nests, 47 (76%) had a mother assigned in the field, including the 2 nests for which maternity was narrowed in the field to 2 candidates. Four mothers were assigned as mothers to 2 nests each, so the pool of known or suspected mothers was 45 birds. We genotyped the 45 known or suspected mothers and 396 adult males. We generated an average of 10.7 typed loci bird⁻¹; genotyping averaged across all birds and all 12 loci was 89.5% complete. Amplification difficulties (perhaps partly attributable to faulty lysis buffer in the 2004 field season) necessitated repeated DNA extractions, amplifications, and electrophoreses. On average, it took 1.9 attempts (i.e., injections in ABI 310 Genetic Analyzer) to obtain each single-locus genotype.

Results of maternity check.—By using CERVUS to match chicks with mothers from the genotyped pool, we were able to (1) correct three cases in which a female had been mistakenly assigned

to a nest in the field (in each case, the true mother was not among the females we genotyped); (2) discriminate between candidate mothers in both broods in which field observations had narrowed the potential mothers to 2 females; and (3) assign the correct mother to 3 more broods, even though the mother had not been linked to that nest in the field. One of the 3 “broods” assigned to a mother through genotyping consisted of 3 of the 6 chicks from the 8-egg nest. The other 3 chicks at that nest were not assignable (and the mother of the first 3 chicks was conclusively eliminated, conflicting with those chicks at 3, 4, and 4 loci, respectively). Because it is difficult to observe birds at their nests, and because females were regularly seen in the vicinity of other birds’ nests, we considered mismatches between field assignments and genetic data to represent errors in our field observations rather than cases of intraspecific brood parasitism, although we consider the 8-egg nest the product of 2 females laying in the same nest. Although we had compared all adult female genotypes with all chicks at all sites, the 3 mothers assigned through genetic data to new nests each came from the same 1-ha plot and same year as the nest to which they were assigned. Thus, we had no “false positives” in which a female from a distant marsh was assigned as a mother.

Paternity assignments: Agreement between methods.—The agreement between paternity assignments obtained using CERVUS with those obtained using COLONY was high. Thirty-four families were available for comparison, having had fathers assigned by CERVUS to ≥ 2 chicks. Those families contained 91 assigned chicks and allowed 88 pairwise comparisons. In 83 of the 88 comparisons (94%), COLONY and CERVUS agreed on whether the chicks shared a father. The 5 disagreements came from 5 families and were evenly split between 2 cases in which CERVUS assigned different fathers to a pair of chicks that COLONY considered full siblings and 3 cases in which CERVUS assigned the same father to a pair of chicks that COLONY considered half siblings. We are confident that the results we present give an accurate and unbiased picture of the actual mating system in our population.

Mating patterns of females.—Almost all the females whose broods we sampled had mated with multiple males (Fig. 1). Fifty-seven of 60 broods had multiple paternity, and only nests with 2 or 3 genotyped chicks (all of which were incompletely sampled clutches) had single fathers. Twenty-one of the 60 broods (35%) had a different father for each chick (8 of 10 two-chick broods, 8 of 23 three-chick broods, and 5 of 20 four-chick broods), but mixed paternity with 1 father accounting for >1 offspring was the most common pattern (36 of 60 broods).

The Simpson diversity index (SDI) for all nests in the sample was 0.77, which can be interpreted as the probability that a given pair of chicks in the same nest have different fathers. This measure was consistent across brood sizes (SDI = 0.80, 0.75, 0.80, and 0.69 for brood sizes of 2–5, respectively; $F = 0.43$, $df = 3$ and 56, $P = 0.74$), across years (2003: SDI = 0.76; 2004: SDI = 0.78; $t = 0.35$, $df = 58$, $P = 0.73$), and when we compared the earliest 30 nests in the 2 years combined to the latest 30 nests (SDI = 0.76 and 0.78, respectively; $t = 0.24$, $df = 58$, $P = 0.81$). On average, the minimum number of fathers per brood in all 9 marshes was between 2 and 3 (analysis of variance [ANOVA] comparing marshes: $F = 0.60$, $df = 8$ and 51, $P = 0.778$), with diversity in paternity varying from a low of 0.53 ($n = 2$ nests) at Back River to 0.88 ($n = 5$) at West River (ANOVA: $F = 1.81$, $df = 8$ and 51, $P = 0.097$). Examining paternity patterns

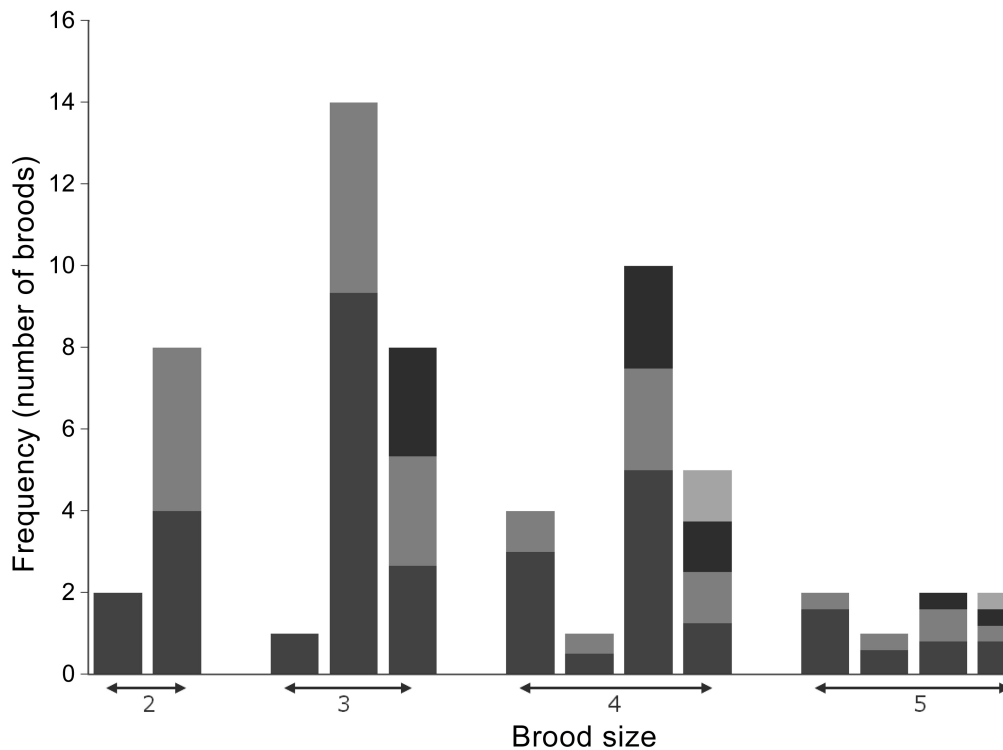


FIG. 1. Patterns of paternity in Saltmarsh Sparrow broods. Bars with a single shading indicate single paternity at a nest. Multiple shadings and their proportions indicate multiple sires and the proportion of young sired by each. Height of the bars indicates the number of broods with a given paternity pattern ($n = 60$). Data from differently sized broods are shown separately. For example, among 20 four-chick broods, 5 had each chick sired by a different father, 10 had 3 fathers accounting for the 4 chicks, and the remainder had 2 fathers accounting for the 4 chicks, most often with 3 chicks from 1 father and 1 from the second. The 6 chicks from the 8-egg nest are here treated as two 3-chick broods (see text).

across nests suggests that the rate of multiple paternity increased with the number of males in the vicinity of the nest, although much variation went unexplained (SDI: $r^2 = 0.24$, $P = 0.006$; minimum number of males per brood: $r^2 = 0.12$, $P = 0.057$; $n = 30$). There was no evidence that multiple paternity rates varied with nesting synchrony (SDI: $r^2 < 0.01$, $P = 0.811$; minimum number of males per brood: $r^2 = 0.01$, $P = 0.401$; $n = 60$).

Do males sire chicks in more than one nesting attempt by the same female?—For 7 females, we had blood samples from 2 successive nests in the same year. In two of those cases, COLONY grouped a single chick from the first brood with another chick from the second brood as full siblings, which implies that the same male fathered chicks with the same mother on two occasions. However, those full sibships were not confirmed by CERVUS, which in each case assigned a father to one but not the other of the chicks. Thus, we have only limited evidence for repeat paternity across broods.

With whom do males mate?—Our data do not present as clear a picture of mating patterns in males as of those in females. Unlike females, which are linked to individual nests, *a priori* information on which offspring a male fathered are lacking. Moreover, the layout of our sample plots, noncontiguous 1-ha plots within a larger marsh, ensures that our sample includes only partial home ranges for most males. We also lack detailed information about male movement patterns across a season, or “residence

times” of males in the area where they were banded. Despite these difficulties, CERVUS assigned 67 of the 396 genotyped males (17%) as fathers to 109 of the 206 chicks (53%). Forty-one males were assigned as fathers to only 1 chick; of the other 26 males, 16 were assigned 2 chicks, 4 were assigned 3 chicks, and 6 were assigned 4 chicks. Of the 26 males assigned to >1 chick, 11 had all of their paternity in 1 nest (9 males sired 2 chicks in a nest, and 2 sired 4 chicks), 14 males sired chicks in 2 nests, and 1 male sired chicks in 3 nests.

Ninety-four of the 109 chicks with known fathers (86%) were the offspring of males banded within 0.5 km of the natal plot (55 were from the same 1-ha plot). Thirteen other chicks (12%) were fathered by males banded at plots between 0.5 and 1.0 km distant. Two chicks (2%) were fathered by males banded at 1.06 and 1.37 km from the nest, which is close to the longest possible distance between plots within the same marsh. In all cases in which males sired chicks in multiple nests, those nests were within 0.55 km of each other, and in 10 of 15 cases the nests were in the same 1-ha plot.

DISCUSSION

We have documented an unusual mating system in Saltmarsh Sparrows that is characterized by extreme levels of multiple mating by females, as well as multiple mating by males. All fully

sampled broods in our study had multiple fathers; on average, broods were the product of ≥ 2.5 fathers; and in a third of broods, every chick had a different father. By way of comparison, in socially monogamous species, multiple paternity is found in less than a fifth of broods on average (Griffith et al. 2002). Two other avian species are known to approach the level of multiple paternity seen in our study: the Greater Vasa Parrot and Superb Fairy-wren (*Malurus cyaneus*). Ekstrom et al. (2007) found that 17 of 17 Greater Vasa Parrot broods (average brood size = ~ 5) had multiple paternity, and 8 of 13 broods with ≥ 3 nestlings genotyped had ≥ 3 fathers. Superb Fairy-wren broods (modal clutch size = 3 eggs) had multiple paternity in 38 of 40 broods (Mulder et al. 1994). We do not have data to calculate a diversity metric for an unbiased comparison with those species, and in the case of the parrot such data are not available (T. Birkhead pers. comm.), but it is very likely that one of these three species has the most promiscuous female birds known. Interestingly, the social breeding systems of these three species differ dramatically. Saltmarsh Sparrows have no pair bonds and no male participation in the care of young, whereas female Greater Vasa Parrots are fed by multiple males during nesting attempts and female Superb Fairy-wrens form pair bonds with a male and often breed in social groups that include other males as helpers, with all males in a group assisting in the rearing of young (Mulder et al. 1994, Ekstrom et al. 2007).

Breeding systems have proved evolutionarily labile in various bird groups, including warblers in the genus *Acrocephalus*, corvids, and shorebirds (Leisler et al. 2002, Ekman and Ericson 2006, Székely et al. 2006). The presence of abundant resources for provisioning young, which potentially emancipates some adults from parental care, is thought to be an important factor allowing the evolution of nonmonogamous mating systems (Emlen and Oring 1977). Salt marshes seem to provide just such resources, given that female Saltmarsh Sparrows have been found to be as productive as pairs of Seaside Sparrows (*Ammodramus maritimus*) with biparental care in the same marshes, with no apparent survival costs (Post and Greenlaw 1982). Various hypotheses have been proposed to explain variation in paternity patterns among birds (summarized by Griffith 2007). This body of work raises a number of predictions about Saltmarsh Sparrows. For instance, if male density influences mating patterns, one would predict that females from smaller marshes will have fewer partners because small marshes tend to support lower densities (Benoit and Askins 2002, C. Elphick unpubl. data). Our data support this connection between male density and female multiple mating. Similarly, if breeding synchrony promotes multiple mating (Stutchbury and Morton 1995), one would predict a link to tidal flooding, which synchronizes nesting by causing extensive nest flooding (Shriver et al. 2007, Gjerdrum et al. 2008b). Our data do not support a connection between nesting synchrony and female multiple mating.

Factors that promote extreme promiscuity are less well understood, partly because there are fewer known species to provide comparisons. Saltmarsh Sparrows share important features of their breeding system with lekking species: no pair bonds and no male parental care, with males supplying only sperm (Greenlaw and Rising 1994). Although the traditional definition of a lek involves a small communal display ground where males gather (Oring 1982), and although lekking species are generally known

for their elaborate displays, broader definitions include exploded leks (Bradbury 1981), where males may display at some distance from each other, and “hidden leks,” a proposed explanation for clumped breeding territories in some socially monogamous species (Wagner 1998). Under some of these definitions, Saltmarsh Sparrows might be considered lekking birds. Although genetic data are available for few lekking species, those species that have been studied vary from having extremely limited or no multiple mating by females (Alatalo et al. 1996), to moderate levels of multiple paternity (Lancot et al. 1997, Semple et al. 2001), to high levels of multiple paternity (Lank et al. 2002, Thuman and Griffith 2005). Thus, it seems that lek breeding systems, as such, do not favor a particular mating pattern and are not useful in explaining extreme multiple mating.

One species stands out as remarkably similar to the Saltmarsh Sparrow: the Aquatic Warbler (*Acrocephalus paludicola*). Not only is each species' breeding system similar—with home-range overlap by males, lack of territoriality, and uniparental care by females—but the outcome is also similar: high multiple paternity, with 60% of Aquatic Warbler nests having at least 2 fathers and some having as many as 4 (Schaefer et al. 2000, Dyrce et al. 2002). The Saltmarsh Sparrow and the Aquatic Warbler are both promiscuous species that lie within clades of mostly monogamous species (albeit with some extrapair fertilization: Murray 1969, Leisler et al. 2002, Hill and Post 2005, Klicka and Spellman 2007). The two species also share similar habitats: sedge (*Carex* spp.) “fen mires” for the warbler, grassy salt marshes dominated by *Spartina* spp. for the sparrow, each flat with “dense monolayers” (Schaefer et al. 2000) of vegetation < 1 m high, over frequently flooded soil.

The level of multiple paternity that we observed implies that females are either unable to choose or uninterested in choosing a single best male to sire their young. Either result would be interesting, and pursuing the mechanics of male–female interactions has been advocated as a way to understand the origins of different mating systems (Westneat and Stewart 2003). At this point, however, descriptions of the mechanics of the Saltmarsh Sparrow's mating system seem fragmentary and contradictory. Greenlaw and Rising (1994) characterized the mating system as scramble-competition polygyny, thus emphasizing male efforts to contact females over female choice and male–male contests. Greenlaw and Rising (1994) also stated that males force copulations. Woolfenden (1956), however, emphasized male–male fights that precede mating, which are perhaps related to males attempting to disrupt the copulation success of other males, a tactic that can affect mating success in other species (Trail 1985). Woolfenden (1956) described a single observed copulation in some detail without suggesting that males force copulations. Although sparrows are particularly hard to observe in saltmarsh habitat, we have seen no strong evidence of forced copulations at our study sites. Moreover, it is worth noting that forced copulations in some bird species have been shown to be remarkably ineffective in securing fertilizations (Dunn et al. 1999). Further study may clarify both male and female roles in Saltmarsh Sparrow breeding and whether those roles vary among populations. In particular, it will be of interest to determine how passive or active females are in mating and whether they actively choose to mate with a diversity of males, perhaps to encourage sperm competition or avoid intragenomic conflict (cf. Zeh 1997).

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