

## GENETIC EVIDENCE FOR MIXED MATERNITY AT A LARK SPARROW NEST

KEVIN ELLISON, Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2 (current address: Wildlife Conservation Society, 301 North Willson Avenue, Bozeman, Montana 59715); kellison@wcs.org

JEREMY D. ROSS, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403; rossjd@bgsu.edu

JUAN L. BOUZAT, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403; jbouzat@bgsu.edu

**ABSTRACT:** Among passerines not nesting in cavities, mixed maternity within a clutch is rare and has been recorded at <1% of the nests of four North American species. Using genetic methods, we report the first case of mixed maternity in the Lark Sparrow (*Chondestes grammacus*). Within an unusually large clutch in Texas, we found that the attending female shared the same mitochondrial haplotype with an unhatched egg and one nestling, but not with another nestling. Analysis with microsatellite DNA confirmed mixed maternity at the nest; the female and a nestling did not share alleles at five of nine loci analyzed. Various behaviors may lead to mixed maternity, including intraspecific reuse of nests, conspecific usurpation, and conspecific parasitism. Our study stresses the important roles that intensive nest monitoring and genetic tests can play in detecting cryptic reproductive strategies.

Mixed maternity at nests is rarely reported among passerines (Yom-Tov 2001, Lyon and Eadie 2008). However, mixed maternity may be common but underdetected in certain species or populations; such a context could alter our interpretation of evolutionary adaptations such as egg recognition, nest defense, and parenting behavior (Yom-Tov 2001). For instance, Latif et al. (2006) only recently recorded the first evidence of conspecific nest parasitism and egg ejection in the Song Sparrow (*Melospiza melodia*) despite widespread study of that species' nesting over the past century (see Smith et al. 2006). In addition to nest parasitism, multiple maternity at a nest can also arise via several mechanisms (see review in Lyon and Eadie 2008): nest reuse (i.e., when a female lays among eggs left in an abandoned nest), errors in nest ownership—possibly paired with the need to lay (Harms et al. 1991), mate change during laying (Griffith et al. 2004), or usurpation (i.e., the forcible takeover of an active nest by a female other than the owner) (Sullivan et al. 1989; reviewed by Ellison 2008).

The Lark Sparrow (*Chondestes grammacus*) appears unusual in its propensity to reuse inactive nests constructed by other individuals (McNair 1984, 1985). Lark Sparrows also attempt to use active nests of other birds, sometimes resulting in successful usurpation of nests of the same or other species (McNair 1984, Ellison unpubl. data). Detecting such mixed maternity at a nest requires intensive monitoring, often encompassing the use of egg marking, video surveillance, or genetic testing (Latif et al. 2006). Clutches that exceed twice the species' mean clutch size can alert researchers to the possibility of conspecific nest parasitism (Yom-Tov 1980). The mean number of eggs Martin and Parrish (2000) reported for the Lark Sparrow is  $3.84 \pm 0.70$  ( $n = 209$  nests). Among 200 nests of the Lark Sparrow (Ellison et al.

2006), we encountered one with seven eggs and therefore suspected that multiple females had used it. To determine if there was evidence for mixed maternity in this nest, we examined mitochondrial DNA sequences and nuclear genotypic data of the adult female, eggs, and nestlings. We hypothesized usurpation, reuse, or intraspecific parasitism as possible explanations for any mixed maternity, and we considered the likelihood of each in light of the physical and genetic evidence.

## METHODS

The Lark Sparrow nest was found on 28 April 2002 in Kinney Co., Texas. It was on the ground, under an overhanging pad of prickly pear (*Opuntia* spp), and contained four eggs when discovered. Two days later, the nest held five Lark Sparrow eggs and we found another two eggs 25 and 38 cm outside the nest. We collected one egg outside the nest; the other was damaged and discarded in the field. On 11 May, we observed five hatchlings were in the nest, and on 13 May we captured an adult female at the nest, banded her, and took a blood sample. This banded female was then observed carrying food to the nest and fledglings through 22 May. On 14 May we banded and sampled the blood of two nestlings (A and B); the third remaining nestling (C) was too small to be banded or bled. On 18 May A and B (the only young we detected) had fledged; we recaptured B and sampled its blood again. We therefore had samples from a nesting female, one egg, and two young from this nest.

Blood samples were stored in Queen's lysis buffer (Seutin et al. 1991) at 4°C. The egg was stored in a sealed bag at -20 °C. To prepare the egg for DNA extraction, we removed the shell while the egg was still frozen and sealed it in a 1.5-mL microcentrifuge tube. We thawed each egg's contents separately and checked for any evidence of a developing embryo. DNA from blood and eggshell samples was extracted by a proteinase-K digestion followed by a standard phenol-chloroform extraction protocol (Sambrook et al. 1989, Strausberger and Ashley 2001). To check against possible contamination we concurrently extracted DNA from an unrelated Lark Sparrow from Nebraska, which we used as a positive control, and performed a blank extraction as a negative control.

Using primer set L16743 (Tarr 1995) and H920 (5'-GTC CCG CAA CCA TTA CAC TA-3'; Ross 2011), we amplified 910 base pairs of the mitochondrial control region (d-loop) from each sample. We purified the products of the polymerase chain reaction by ethanol precipitation and sequenced them with ABI BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA). For sequencing we used the H920 and two additional nested primers, H598 (5'-TTC AAC CGA CCA CTT GTA TCT G-3'; Ross 2011) and L437 (Tarr 1995). We sequenced the gene on an ABI377 Genetic Analyzer (Applied Biosystems) and checked the accuracy of the scoring visually, then exported the sequences to BioEdit (version 7.09, Hall 1999) to check those from the same sample for mismatched base pairs.

We genotyped nine nuclear microsatellites from each sample: *Dpμ16* (Dawson et al. 1997), *Maμ23* (Alderson et al. 1999), *ESCμ1* (Hanotte et al., 1994), *Asμ09* and *Asμ15* (Delany et al. 2000), *Cμ02* (Gibbs et al.

1999), *Pdoμ3* (Griffith et al. 1999), *Gf01b* (Rasner et al. 2004), and *Gf05* (Petren 1998). We had tested these microsatellites previously, finding them to be polymorphic in the Lark Sparrow. The polymerase chain reactions for amplification of the microsatellites followed conditions described by Ross (2011). We genotyped the microsatellites with an ABI377 Genetic Analyzer and scored them with GeneScan software (Applied Biosystems).

RESULTS

Sequences of the mitochondrial d-loop of the adult female banded at the nest, the eggshell, and nestling B were identical (Table 1). That of nestling A was different, with 9 base-pair substitutions in the DNA fragment of 910 base pairs. As expected, the mitochondrial d-loop of the control sample yielded a completely different haplotype (Table 1).

The female shared at least one allele at each microsatellite locus with nestling B, which is consistent with a mother-offspring relationship (Table 1). Also, the female and nestling A did not share any alleles at five microsatellite loci (Table 1). Although we were not able to genotype all the loci for the eggshell, genotypes at four loci (*Maμ23*, *Dpμ16*, *Asμ09*, and *Gf05*) were identical to those of the attending female. Since the embryo in the egg was undeveloped and we extracted the DNA from tissue remaining in the eggshell, we can assume that the genotypes of the eggshell correspond to those of the mother. Neither the blank extraction nor the negative controls of the polymerase chain reaction yielded any product. Thus we are confident that the products of DNA amplification from the eggshell were not from contamination.

**Table 1** Mitochondrial DNA Haplotypes and Microsatellite Genotypes of the Attending Female Lark Sparrow, Two Young Sampled in the Nest, and the Shell of an Egg

	Female	Nestling A	Nestling B	Eggshell	Nebraska sample
mtDNA haplotype <sup>a</sup>	H1	H2	H1	H1	H3
Microsatellite genotypes <sup>b</sup>					
<i>Dpμ16</i>	156/158	<b>158</b> /160	<b>156/158</b>	<b>156/158</b>	<b>158</b> /160
<i>Maμ23</i>	143/145	141/ <b>143</b>	<b>143/145</b>	<b>143/145</b>	<b>143</b> /143
<i>ESCu1</i>	169/179	129/155	151/ <b>179</b>	—	127/127
<i>Asμ09</i>	133/133	<b>133/133</b>	129/ <b>133</b>	<b>133/133</b>	129/ <b>133</b>
<i>Asμ15</i>	121/143	131/145	<b>143/143</b>	—	119/133
<i>Cuμ02</i>	112/112	<b>112/112</b>	<b>112/112</b>	—	110/ <b>112</b>
<i>Pdoμ3</i>	91/91	83/147	<b>91/91</b>	—	119/119
<i>Gf01b</i>	216/224	220/222	<b>216/216</b>	—	<b>216</b> /220
<i>Gf05</i>	185/185	187/187	<b>185/185</b>	<b>185/185</b>	<b>185</b> /189

<sup>a</sup>Haplotypes H1, H2, and H3 correspond to Genbank sequences FJ348339, FJ348340, and FJ348341.

<sup>b</sup>Alleles of microsatellite genotypes are reported in number of base pairs; those matching the attending female are in bold.

## DISCUSSION

As far as is known, mixed maternity in nests of North American passerines is rare. For cup-nesting species, conspecific parasitism or usurpation has been previously noted in 10, the Cave Swallow (*Petrochelidon fulva*), Cliff Swallow (*P. pyrrhonota*), Hooded Warbler (*Setophaga citrina*), Song Sparrow, Field Sparrow (*Spizella pusilla*), Yellow-eyed Junco (*Junco phaeonotus*), Red-winged Blackbird (*Agelaius phoeniceus*), Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*), Brewer's Blackbird (*Euphagus cyanocephalus*), and Bullock's Oriole (*Icterus bullockii*), though at relatively few nests (Sullivan et al. 1989, Petter et al. 1990, Harms et al. 1991, Arnold and Owens 2002, Latif et al. 2006). Our genetic analyses revealed that an attending female Lark Sparrow was not related to at least one nestling (nestling A) at a nest where she was the biological mother of nestling B and an egg found outside the nest (Table 1).

The mixed maternity of the clutch may reflect intraspecific reuse of the nest, as the nest could have been abandoned with one or more eggs in it when a second female took over the nest. Indeed, birds are more apt to abandon nests during laying when the parental investment in the nest is low and the benefit of choosing a more desirable nest site may outweigh the costs of abandonment (Hosoi and Rothstein 2000). However, this conclusion implies that the female that reused the nest had ejected at least one of her own eggs, since the genetic analyses revealed that the egg found outside the nest was laid by the nesting female.

Mixed maternity of the clutch may also have arisen through usurpation; that is, the banded female may have usurped the nest, with one or more unrelated eggs within it (Lindell 1996). Attempted usurpation followed by the banded female regaining her nest and raising unrelated young alongside her own is equally likely. Under this scenario, a would-be usurper ejected part of the host's clutch, laying at least one egg of her own (nestling A). We cannot say how many of the three new eggs in the nest were laid by either female. Nevertheless, the banded female raised at least one related and one unrelated young.

Mixed maternity at the nest could have also occurred as a result of conspecific parasitism. We are unable to distinguish conspecific usurpation and nest parasitism as each can result in the same outcome. We do note that, in several instances, Lark Sparrows have raised unrelated young, including those of other species. This occurred despite some nests containing eggs of the previous owner (McNair 1984, Ellison unpubl. data). Further study is needed to determine if Lark Sparrows can eject eggs. Peer et al. (2000) reported that they rejected 2 of 3 undersized non-mimetic eggs but appear unable to discriminate undersized mimetic model eggs, as they accepted 2.

Ross (2011) closely monitored approximately 75 color-banded Lark Sparrows in Ohio. In each of three years, both females and males defended territories from conspecific invaders of both sexes. None of 14 nests known to be built by marked females was permanently usurped. On three occasions (in 51 territories studied in 2006 and 2007), however, when a fledgling barely capable of flight was flushed and produced an alarm call, it was defended by three adults, two being the juvenile's social parents and the third an unbanded adult of unknown sex. These observations might signal

## GENETIC EVIDENCE FOR MIXED MATERNITY AT A LARK SPARROW NEST

a parasitic/usurping female offering parental support to what could be her offspring. As Lark Sparrows apparently saturated the suitable habitat at this site, females may have adopted strategies that allowed them to breed where no territories were available.

In summary, we report here the first case of mixed maternity in a wild population of the Lark Sparrow. We cannot determine how the eggs of two females came to be in the same nest. Regardless, our observation reflects a behavior of interest and should serve as an added impetus for the consideration of alternative reproductive tactics that may be revealed by intensive monitoring of nests.

### ACKNOWLEDGMENTS

We thank the land owners of Fort Clark Springs for the permission to study the Lark Sparrow on their property and the Ohio Department of Natural Resources for financial support. We also thank Mike D. Boyd for his assistance in the field.

### LITERATURE CITED

- Alderson, G., Gibbs, H. L., and Sealy, S. G. 1999. Parentage and kinship studies in an obligate brood parasitic bird, the Brown-headed Cowbird (*Molothrus ater*), using microsatellite DNA markers. *J. Heredity* 90:182–190.
- Arnold, K. E., and Owens, I. P. F. 2002. Extra-pair paternity and egg dumping in birds: Life history, parental care and the risk of retaliation. *Proc. Royal Soc. London B* 269:1263–1269.
- Dawson, R. J. G., Gibbs, H. L., Hobson, K. A., and Yezzerinac, S. M. 1997. Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the Yellow Warbler), and their use in population studies. *Heredity* 79:506–514.
- Delany, M. F., Geisel, J. T., and Brazeau, D. A. 2000. Genetic variability among populations of the Florida Grasshopper Sparrow. *J. Wildlife Mgmt.* 64:631–636.
- Ellison, K. 2008. Nest reuse by Vermilion Flycatchers in Texas. *Wilson J. Ornithol.* 120:239–244.
- Ellison, K., Sealy, S. G., and Gibbs, H. L. 2006. Genetic elucidation of host use by individual sympatric Bronzed Cowbirds (*Molothrus aeneus*) and Brown-headed Cowbirds (*M. ater*). *Can. J. Zool.* 84:1269–1280.
- Gibbs, H. L., Tabak, L. M., and Hobson, K. 1999. Characterization of microsatellite loci for a neotropical migrant songbird, the Swainson's Thrush (*Catharus ustulatus*). *Molec. Ecol.* 8:1151
- Griffith, S. C., Stewart, I. R. K., Dawson, D. A., Owens, I. P. F., and Burke, T. 1999. Extra-pair paternity in mainland and island populations of a socially monogamous bird, the House Sparrow (*Passer domesticus*): Is there an "island effect"? *Biol. J. Linnean Soc.* 68:303–316.
- Griffith, S. C., Lyon, B. E., and Montgomerie, R. 2004. Quasi-parasitism in birds. *Behav. Ecol. Sociobiol.* 56:191–200.
- Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids. Symp. Ser.* 41:95–98.
- Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A. and Burke, T. 1994. Isolation and characterization of microsatellite loci in a passerine bird: The Reed Bunting *Emberiza schoeniclus*. *Molec. Ecol.* 3:529–530.
- Harms, K. E., Beletsky, L. D., and Orians, G. H. 1991. Conspecific nest parasitism in three species of New World blackbirds. *Condor* 93:967–974.
- Hosoi, S.A., and Rothstein, S. I. 2000. Nest desertion and cowbird parasitism: Evidence for evolved responses and evolutionary lag. *Anim. Behav.* 59:823–840.

## GENETIC EVIDENCE FOR MIXED MATERNITY AT A LARK SPARROW NEST

- Latif, Q. S., Grenier, J. L., Heath, S. K., Ballard, G., and Hauber, M. E. 2006. First evidence of conspecific brood parasitism and egg ejection in Song Sparrows, with comments on methods sufficient to document these behaviors. *Condor* 108:452–458.
- Lindell, C. 1996. Patterns of nest usurpation: when should species converge on nest niches? *Condor* 98:464–473.
- Lyon, B. E., and Eadie, J. M. 2008. Conspecific brood parasitism in birds: A life-history perspective. *Annu. Rev. Ecol. Evol. Syst.* 39:343–363.
- Martin, J. W., and Parrish, J. R., 2000. Lark Sparrow (*Chondestes grammacus*), in *The Birds of North America* (A. Poole and F. Gill, eds), no. 488. Birds N. Am., Inc., Philadelphia.
- McNair, D. B. 1984. Reuse of other species nests by Lark Sparrows. *Southwest. Nat.* 29:506–509.
- McNair, D. B. 1985. A Comparison of oology and nest record card data in evaluating the reproductive biology of Lark Sparrows, *Chondestes grammacus*. *Southwest. Nat.* 30:213–224.
- Peer, B. D., Robinson, S. K., and Herkert, J. R. 2000. Egg rejection by cowbird hosts in grasslands. *Auk* 117:892–901.
- Petren, K. 1998. Microsatellite primers for *Geospiza fortis* and cross-species amplification in Darwin's finches. *Molec. Ecol.* 7:1782–1784.
- Petter, S. C., Miles, D. B., and White, M. M. 1990. Genetic evidence of mixed reproductive strategy in a monogamous bird. *Condor* 92:702–708.
- Rasner, C. A., Yeh, P., Eggert, L. S., Hunt, K. E., Woodruff, D. S., and Price, T. D. 2004. Genetic and morphological evolution following a founder event in the Dark-eyed Junco, *Junco hyemalis thurberi*. *Molec. Ecol.* 13:671–681
- Ross, J. D. 2011. The evolutionary history, demographic independence and conservation status of two North American prairie bird species: The Greater Prairie-chicken and the Lark Sparrow. Ph.D. dissertation, Bowling Green State University, Bowling Green, OH.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Seutin, G., White, B. N., and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* 69: 82–90.
- Smith, J. N. M., Keller, L. F., Marr, A. B., and Arcese, P. 2006. Conservation and biology of small populations: The Song Sparrows of Mandarte Island. Oxford University Press, New York.
- Strausberger, B. M., and Ashley, M. V. 2001. Eggs yield nuclear DNA from egg-laying cowbirds, their embryos and offspring. *Cons. Genetics* 2:385–390.
- Sullivan, K. A., Cole, J., and Villalobos, E. M. 1989. Intraspecific nest usurpation by a Yellow-eyed Junco. *Wilson Bull.* 101:654–655.
- Tarr, C. L. 1995. Amplification and determination of mitochondrial control-region sequences in oscine passerines. *Molec. Ecol.* 4:527–529.
- Yom-Tov, Y. 1980. Intraspecific nest parasitism in birds. *Biol. Rev.* 55:93–108.
- Yom-Tov, Y. 2001. An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis* 143:133–143.

Accepted 9 March 2013