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Documentation of a hybrid *Spizella* sparrow using spectrographic and genetic analysis

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SUMMARY.—We report the first well-documented hybrid between Field *Spizella pusilla* and Clay-coloured Sparrows *S. pallida*. This hybrid combination has previously been suggested from field observations in eastern North America, but not confirmed. We encountered an individual in Lorain County, Ohio, USA, during surveys for the state's second breeding bird atlas. It was a territorial male, singing a buzzy but accelerating trilled song with characteristics of both parents. It responded vigorously to playback of both Field and Clay-coloured Sparrows. In the hand, the bird showed plumage characteristics of both species and intermediate measurements. Using molecular data, we were able to confirm Clay-coloured Sparrow as the mother of the individual, with support for Field Sparrow as the father. This and other recent field observations of this hybrid pairing have been reported at the eastern boundary of the expanding breeding range of Clay-coloured Sparrow, suggesting that this hybrid combination can be expected elsewhere in the Great Lakes region and in New England, where these species are increasingly syntopic.

The six species of sparrows in the genus *Spizella* are common in open habitats across North America. Clay-coloured Sparrow *S. pallida* occurs in central North America, in shrubland, grassland and prairies, throughout the year (Grant & Knapton 2012). Field Sparrow *S. pusilla* is found in brushy pasture and grassland, and second-growth scrub, in eastern North America, also year-round (Carey *et al.* 2008). Both species are migratory, with overlap in their breeding ranges in the Great Lakes region, and in migration and winter in the eastern Great Plains as far south as Texas. There have been several field observations of possible hybridisation behaviour and putative hybrids, but confirmation has been lacking. Here we describe a hybrid between these species, with supporting vocal, morphological and genetic data.

Methods

Encounter details.—The putative hybrid was first seen by EK on 3 July 2008 while undertaking field work for the second Ohio Breeding Bird Atlas project (Rodewald *et al.* 2016). The bird was singing in a small grassland at the Charlemont Reservation of Lorain County Metro Parks, Lorain County, Ohio, USA (41°07.469'N, 82°26.906'W). The bird resembled a Clay-coloured Sparrow, a very rare breeder in the state (Peterjohn 2001), but some coloration and song details were inconsistent with the species. A brief recording of its song was made by ATB using a Kodak DX7440 digital camera on 11 July 2008.

On 16 July 2008, ATB & AWJ returned to the site at 07.15 h. The bird was still singing continuously, and we set up mist-nets in the centre of the bird's apparent territory, based on repeated use of song perches. We initiated playback of a typical Clay-coloured Sparrow

song on a speaker below the net. The bird immediately approached the speaker and spent ten minutes counter-singing against the playback while perched on higher stems. This behaviour occurred within 5 m of the playback equipment, but the bird did not come closer to the mist-nets. We then changed the playback to a typical Field Sparrow song, and the bird immediately flew directly at the speaker and was caught in the mist-net. It was measured and photographed, with several contour feathers and one secondary feather collected before the bird was released.

Genetic analyses.—Plucked feathers were stored in a clean 2 mL tube in the field, then refrigerated at 4°C at the Cleveland Museum of Natural History. Feathers were extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA), with 30 µL of DTT (Dithiothreitol) added to the initial digestion step to digest the feather. We also extracted DNA, using standard protocols, from the other widespread North American species of genus *Spizella* for comparison (Table 1). We amplified two genes using the polymerase chain reaction (PCR); the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) was amplified using primers and conditions from Drovetski *et al.* (2004), and the ninth intron of the nuclear-encoded aconitase 1 gene (ACO1-19) was amplified using primers and conditions from Barker *et al.* (2008). The PCR results were cleaned and sequenced at the CWRU Genomics Center (Cleveland, OH) using BigDye Terminator 1.1 and 3.1 Cycle sequencing kits (Applied Biosystems, Foster City, CA) on an ABI 3730 Genetic Analyzer using the same sets of primers. Sequences were aligned in Geneious version 9.0 (Kearse *et al.* 2012), and aligned and compared to several GenBank sequences of congeners (Table 1).

Results

Morphology and song.—Plumage features were intermediate vs. both putative parental species (Fig. 1). All bare parts—the bill (including gape and mouth lining), legs, feet, and toes—were pinkish orange, resembling a Field Sparrow, but with a dusky tip to the culmen like a Clay-coloured Sparrow. The hybrid had a weak white eye-ring. The grey ear-coverts contrasted with a pale supercilium and the pale malar. The lores were pale brown, and the crown was streaked rusty, with a subtle central crown-stripe. The nape was grey, and the back was streaked black, brown and rusty.

Unflattened wing chord was 65.5 mm; Pyle (1997) reported a range of 61–72 mm for Field Sparrow and 59–67 mm for Clay-coloured Sparrow. The tail was 64 mm. Pyle

TABLE 1
Museum specimen data and GenBank accession numbers for DNA sequences used in this study. Museum acronyms: CMNH = Cleveland Museum of Natural History; UMMZ = University of Michigan Museum of Zoology, Ann Arbor.

Taxon	ND2 GenBank	ACO9 GenBank	Specimen number	Collector number
<i>S. pallida</i>	FJ547320	n/a		
<i>S. pallida</i>	MK530936	MK530941	UMMZ 242717	n/a
<i>S. pusilla</i>	EF529824	KC007881		
<i>S. pusilla</i>	MK530938	MK530942	CMNH 74050	CLB 949
<i>S. passerina</i>	FJ547319	n/a		
<i>S. passerina</i>	MK530937	MK530943	CMNH 73488	CLB 782
<i>S. breweri</i>	AF290121	n/a		
<i>S. breweri</i>	MK530939	MK530944	CMNH 73031	CLB 489
<i>S. pallida</i> × <i>S. pusilla</i>	MK530940	MK530945	n/a	n/a



Figure 1. Hybrid Field *Spizella pusilla* × Clay-coloured Sparrow *S. pallida* mist-netted in Charlemont Reservation, Lorain County, Ohio, 16 July 2008; the bill and legs have a pink tone resembling Field Sparrow, but plumage characters are intermediate, with a pale malar that is absent in Field Sparrow and rusty tones throughout the body, which are absent in Clay-coloured Sparrow (Andrew W. Jones)

(1997) reported a range of 59–74 mm for Field Sparrow and 55–63 mm for Clay-coloured Sparrow. We identified the bird as an after-second-year adult, based on the relatively small amount of wear on the broad tips of the rectrices, a lack of moult limit in the primaries and secondaries, and the broad shape and minimal wear to the alula and primary-coverts. These ageing criteria are common to both parental species (Pyle 1997).

Two songs were recorded by ATB during his visit on 11 July 2008. These had a buzzy tone that was similar to a typical Clay-coloured Sparrow. The song was an extended introductory note followed by a series of notes on the same relative frequency but decreased in length, and with a decrease in time between each note (Fig. 2).

Genetic analyses.—There were 134 variable sites within the ND2 data among the four species of *Spizella*, including the hybrid. Within these sites, the hybrid differed from the two Clay-coloured Sparrow sequences at just three loci. Pairwise differences between the hybrid and the other species were as follows: Clay-coloured Sparrow: 0.2 and 0.4%, Chipping Sparrow *S. passerina*: 8.8%, Brewer's Sparrow *S. breweri*: 8.5% for both, and Field Sparrow: 7.9 and 8.0%. Within the ACO1-19 data, there were three variable sites among Clay-coloured, Field and the hybrid sparrow. Two loci had singleton alleles restricted to one of the two Field Sparrow samples and were therefore uninformative in determining parentage of the hybrid. The third variable locus had a fixed difference between the Clay-

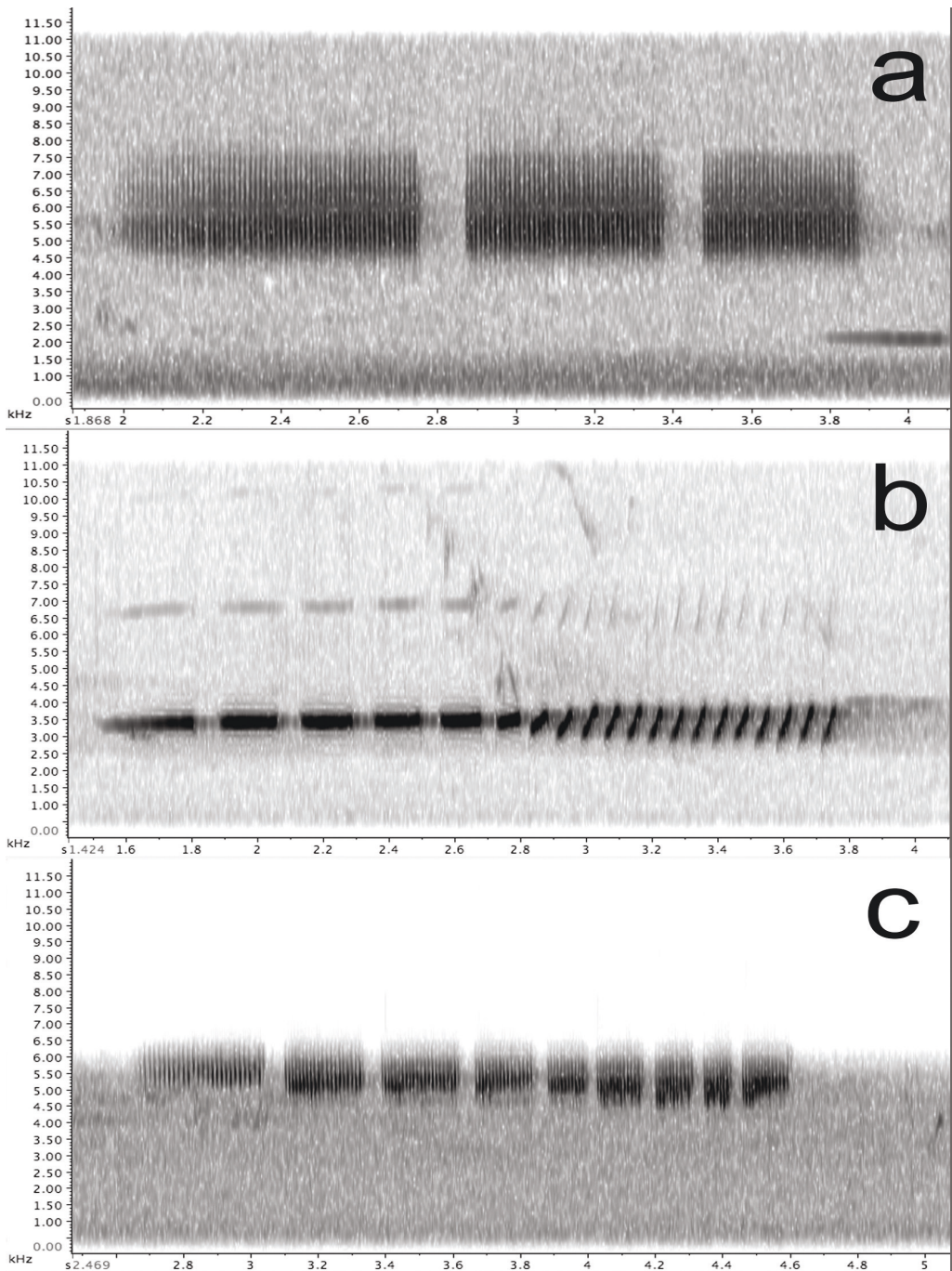


Figure 2. Sonograms of song from three *Spizella* sparrows. A: Clay-coloured Sparrow *S. pallida*, recorded in Oscoda County, Michigan, by A. Chartier on 27 June 2008 (xeno-canto.org, XC31304). B: Field Sparrow *S. pusilla*, recorded in Grant County, Wisconsin, by P. Marvin on 21 May 2011 (XC167935). C: Field × Clay-coloured Sparrow hybrid, recorded in Lorain County, Ohio, by A. T. Boone on 11 July 2008 (Macaulay Library ML113644741). The hybrid individual's song has the buzzy tones of a Clay-coloured Sparrow, but the accelerating cadence of a Field Sparrow.

coloured and Field sparrow samples, with all samples being homozygous. The hybrid was heterozygous, carrying one Clay-coloured Sparrow allele and one Field Sparrow allele. The small amount of DNA that we extracted from the feathers was used up in the ND2 and ACO1-19 PCR reactions, so no additional nuclear loci were available.

Discussion

The combination of intermediate song characteristics, aggression to both parental songs, plumage appearance, bare-part colours, intermediate measurements, and a heterozygous nuclear allele confirm that this bird was a hybrid between a male Field Sparrow and a female Clay-coloured Sparrow.

Field and Clay-coloured Sparrow hybridisation has been suggested by multiple observers in the literature and eBird reports (Sullivan *et al.* 2009). Nests have been reported with both species attending them in Allegany County, New York (Brooks 1980) and Holmes County, Ohio (Weaver 2002). An adult that was thought to be a Clay-coloured × Field Sparrow hybrid was reported in Grand Isle County, Vermont, with intermediate plumage and song characteristics matching the present individual (Hoag 1999). Two additional reports are available at eBird with photographs of adults that are very similar to the present case, but with more rusty tones on the head and eyestripe, and no information concerning vocalisations (Hunterdon County, New Jersey; <https://ebird.org/view/checklist/S7726349>, accessed 10 August 2018; Muskingum County, Ohio; <https://ebird.org/view/checklist/S45469502>, accessed 10 August 2018). Finally, there are observations from Le Haut-Saint-Laurent County, Quebec, Canada, where an apparent hybrid male successfully fledged offspring with a female Clay-coloured Sparrow. The apparent hybrid sang a pure Clay-coloured Sparrow song, and resembled a pure Clay-coloured Sparrow except for rusty tones in the crown and supercilium, and a pink bill. This individual was perhaps a backcross hybrid (L. Tremblay pers. comm., <https://ebird.org/view/checklist/S46915359>, <https://ebird.org/view/checklist/S46943720> and <https://ebird.org/canada/view/checklist/S46980332>, accessed 26 September 2018).

Most studies of differential responses to playback involving hybrid birds are directed at patterns of response across hybrid zones, with a focus on the two parental species (e.g., Billerman & Carling 2016). In many well-studied systems, song is not strongly differentiated across the hybrid zone (e.g., Pearson & Rohwer 2000, Kenyon *et al.* 2017). Within Emberizidae, one similar rare hybrid case was examined for response intensity. A hybrid between a female White-throated Sparrow *Zonotrichia albicollis* and a male Dark-eyed Junco *Junco hyemalis* was held in captivity and presented with playback; it responded most strongly to its own song, at comparable intermediate levels to both parental species, and least strongly to a control (Jung *et al.* 1994). These studies consistently display aggression to both parental species. In the present case, the hybrid *Spizella* responded more strongly to Field Sparrow song. The hybrid's father was a Field Sparrow, and this species' song is likely the one it would have initially imprinted on as a nestling.

Clay-coloured and Field Sparrows are closely related. Klicka *et al.* (2014) found strong support for a monophyletic genus *Spizella*, with Clay-coloured likely sister to a clade of five species including Field Sparrow. Clay-coloured Sparrow has hybridised with both Brewer's Sparrow (Rotenberry *et al.* 1998) and Chipping Sparrow (Middleton 1998). Field Sparrow has not been documented to hybridise with other species except perhaps Vesper Sparrow *Poocetes gramineus* (Doolittle 1929). Hybridisation between Field Sparrow and Clay-coloured Sparrow may be increasingly common. All reported crosses reviewed here are within the breeding range of Field Sparrow, and coincide with the eastern edge of the Clay-coloured Sparrow range, where the latter species is increasingly observed during the

breeding season (Grant & Knapton 2012). Here, hybridisation between the two taxa may be more likely as edge-of-range individuals lack suitable mates.

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