Methods

DNA from two regions of the mitochondrial genome (control region and ND2 gene) of the two Fair Isle wagtails and the Devon bird was analysed. Several other Yellow Wagtail museum specimens, of subspecies *flavissima*, *flava*, *thunbergi*, *cinereocapilla*, *feldegg*, *tschutschensis* and *taivana*, were also used in the analysis, as controls – all the controls yielded DNA sequences consistent with their stated provenance and subspecies. For all specimens, a 231 base pair (bp) fragment of the mitochondrial control region (CR) was isolated by PCR using the MCRI1 and MCRI2 primers (Ödeen & Björklund 2003), from either one or two toepads (the museum specimens) or the base of a contour feather (the Devon bird). Separately, using the standard L5216 (CCATACCCCGRAAATG) and H6313 primers (ACTCTTRTTTAAGGCTTTGAAGGC), a 1,040-bp DNA fragment encompassing the entire mitochondrial ND2 gene was amplified from the Devon bird. For museum specimens, with more degraded DNA, it was not possible to isolate the full ND2 gene so a new primer, Mota.H5502 GATGTTGGGTGGGTGAGYTG, was designed by Dr Michael Sorenson (Boston University) to isolate a 323-bp fragment in combination with primer L5216. PCR products were purified using the QIAGEN gel extraction kit and sequenced by Source BioScience LifeSciences DNA Sequencing Service. The raw sequence was proofread for errors and ambiguities and then compared to existing sequences from birds of known provenance online using nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

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36–38. The 1909 Fair Isle Yellow Wagtail *Motacilla flava tschutschensis*/*plexa*. The white throat and supercilia are apparent, but the breast and belly are suffused dark buff, greyer on the flanks. The long hind claw is visible.

feathers in the upperparts of the Colyton bird. Given the known variability in wagtail plumages between and within populations (most eastern and western birds would be expected to show olive and yellow plumage hues in first-winter plumage), the identity of these birds is open to question without further evidence. The purpose of this study was to carry out a genetic analysis of all three wagtails to determine whether they were likely to have an eastern origin.

Results

Fair Isle 1909

Both the ND2 gene and the control-region sequences showed unequivocally that this bird was from one of the northeastern taxa (*tschutschensis* or *plexa*). Multiple individuals from all taxa have been sequenced previously at the ND2 gene, and there is excellent discrimination between birds in eastern and western clades. The 323 bp of ND2 gene successfully isolated and sequenced (EMBL Nucleotide Sequence Database accession number HE978843) was >99% identical (1 or 2 bp difference) to multiple

sequences from individuals sampled in the area surrounding the Anabar River, Noyabr'sk, Alaska and the Yamal Peninsula. These locations correspond to the taxa *tschutschensis* and *plexa*. The most similar *macronyx* and *taivana* sequences were 97% identical, and the most similar western Yellow Wagtail taxa showed only 93% similarity. The control-region sequence was not as informative because fewer individuals of fewer taxa were in the database (e.g. up to October 2012, only three *tschutschensis* and no *plexa* have previously been sequenced at this site). The 231 bp of sequence from this bird (accession number HE978840) was >99% identical to the database sequences from *tschutschensis* (1 or 2 bases difference), 97% identical to database sequences of *taivana* and *macronyx* (5 bp difference) and also to samples of White and Citrine Wagtails (5 bp difference), and 93–96% identical (at least 7 bp difference) to western Yellow Wagtails of all subspecies.

Fair Isle 1912

Both the ND2 gene and the control-region sequences showed unequivocally that this bird was from one of the western taxa. The 321 bp of ND2 sequence isolated (accession number HE978844) was 100% identical to multiple database sequences from western taxa including nominate *flava*, *flavissima*, *beema* and *lutea*. The most similar sequence from an eastern taxon (*macronyx*) showed 94% similarity (i.e. 18 bp changes). The 231 bp of control-region sequence from this bird (accession number HE978841) was 100% identical to the database sequences of multiple individual western Yellow Wagtails of six subspecies (*flava*, *flavissima*, *thunbergi*, *iberiae*, *beema*, *cinereocapilla*) and 99% identical (1–2 bp difference) to multiple other individuals of these taxa, and also *lutea*, *feldegg* and *pygmaea*. There were 4 bp differences between this bird and the closest *macronyx* and *taivana*, and the most similar *tschutschensis* was 7 bp different.

Devon 2010

Both analyses placed the Devon bird unambiguously in the 'northeastern' clade. Its full ND2 sequence (1,040 bp, accession number HE978845) was 100% identical to that of specimens collected at Chukotskiy, Magadan and Yakutia (Russia). The first two locations are in the range of *tschutschensis* and the last location is assignable to either *tschutschensis* or *plexa*. The ND2 sequence was 98% identical to sequences from Citrine Wagtails and from individuals of *macronyx* and *taivana* (16 bp changes), effectively ruling out these taxa. The nearest 'Western' Yellow Wagtail taxa showed only 93% sequence similarity. The 231 bp of control region (accession

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39–41. The 1912 Fair Isle Yellow Wagtail *Motacilla flava*. The upperparts are a cleaner grey than those of the 1909 bird. Scattered yellow feathering is visible in the supercilium, throat, breast and belly.