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TECHNICAL NOTE

Characterization of SNP markers for the painted bunting (*Passerina ciris*) and their relevance in population differentiation and genome evolution studies

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Abstract

We developed nuclear single nucleotide polymorphism (SNP) markers for the Painted Bunting (*Passerina ciris*) using next-generation sequencing genotyping on the Illumina HiSeq4000 platform. Following the best-RAD approach, we sequenced 96 individuals from five breeding populations occurring in the United States. We assessed the discerning power of 105,000 SNPs and confirmed the quality of these markers in population differentiation studies. We detected outlier SNPs within or near protein coding genes, including sequences coding for olfactory receptors which may relate to migratory navigation. Finally, we provide a novel list of 33 primer pairs flanking SNPs to link individuals to regional populations and improve our understanding of the evolution of this migratory songbird.

Keywords SNPs development · Painted Bunting · Illumina HiSeq4000 · Protein coding regions · Olfactory receptors · Songbird migration

The Painted Bunting (*Passerina ciris*) is a small North American songbird in the Cardinalidae family and it reproduces along the Atlantic coast of the United States (*P.c. ciris*), where some individuals are year-round residents, and in the southern part of the US (*P.c. pallidior*), where the larger population of the Great Plains is composed by migrants (Lowther et al. 1999; Contina et al. 2013). Considering the remarkable differences between western and eastern Painted Buntings in terms of migratory and molting strategies, suggesting differential selective pressures, and that some populations across the breeding range are steeply declining (Sykes and Holzman 2005; Sykes et al. 2007) the development of high resolution genetic markers that can discern subspecies and individuals from different populations and the identification of candidate genomic regions under selection is warranted (Ruegg et al. 2014; Contina et al. 2016).

We collected blood samples from 96 individuals from five distinct breeding populations occurring in the United States (Fig. 1a). We isolated and purified DNA from each of the 96 samples using Qiagen™ DNeasy Blood and Tissue kit following the manufacturer's instructions and normalized the final DNA concentration of each sample to 75 ng in a 10 µL volume. We implemented the best-RAD protocol (Ali et al. 2015) and used SbfI restriction enzymes (New England Biolabs, NEB) to cut fragments of genomic DNA at specific sites. We implemented sonication on a Bioruptor NGS sonicator (Diagenode) to fragment DNA to ~400 bp

Data Accessibility The data used in this study are accessible at GenBank (probe accessions Pr032826547 - Pr032826579).

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