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Short Communication

Speciational history of North American *Haemorhous* finches (Aves: Fringillidae) inferred from multilocus data

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ABSTRACT

We investigated species relationships and timing of speciation in North American *Haemorhous* finches by using a mitochondrial phylogeographic approach combined with a multilocus species tree reconstruction. *Haemorhous purpureus* and *H. cassinii* were strongly supported as sister taxa, and *H. mexicanus* was sister to *H. purpureus* + *H. cassinii*. Our divergence times indicated that diversification within *Haemorhous* occurred progressively from the Late Miocene into the Pleistocene. Our inferred pattern of speciation demonstrates the complexity of the origins of North American birds, and provides additional evidence that a single cause for speciation in closely related North American birds, such as Late Pleistocene glacial–interglacial cycles, is unlikely.

1. Introduction

North American finches in the genus *Haemorhous* include three species that are collectively distributed throughout the United States and Mexico (Fig. 1). *Haemorhous mexicanus* is distributed from western North American in open and semi-open habitats to urban and suburban habitats in the eastern United States. *Haemorhous cassinii* occurs in western North America in coniferous forests of the Sierra Nevada, Rocky, and Cascade Mountains. *Haemorhous purpureus* inhabits temperate mesic coniferous forests in western North America, and has expanded its distribution east of the Great Plains during the past 150 years (Wang et al., 2003; Hawley et al., 2008). These three species were previously placed in the genus *Carpodacus*. However, several studies suggested that North American *Carpodacus* were not closely related to any of the 23 Old World species in the genus (Arnaiz-Villena et al., 2007; Zuccon et al., 2012). Since this finding rendered *Carpodacus* as polyphyletic, the three North American species were assigned to the genus *Haemorhous* (Chesser et al., 2012). Phylogenetic relationships among North American *Haemorhous* finches have been inferred from allozyme data (Marten and Johnson, 1986), mitochondrial DNA (Arnaiz-Villena et al., 2007), and multilocus data (Lerner et al., 2011; Zuccon et al., 2012), but no studies to date have used a multilocus phylogeographic approach that included all three species.

Phylogenetic and phylogeographic studies on North American birds have played a central role in understanding the impact of Pleistocene glacial–interglacial cycles on rates of speciation (Klicka and Zink, 1997; Johnson and Cicero, 2004; Zink et al., 2004). A number of phylogeographic studies on North American birds have found complex spatial and temporal patterns of diversification across multiple habitat types (e.g., montane highlands: Manthey et al., 2011; McCormack et al., 2011; van Els et al., 2012; Walstrom et al., 2012; mixed forest habitats: Klicka et al., 2011; scrub, edge, and non-forest habitats: Smith et al., 2011). The discordance in evolutionary histories observed in North America birds highlights the need to incorporate additional data from codistributed birds to better our understanding of how bird assemblages across the continent responded to Pleistocene glacial cycles. The goal of our study was to use a multilocus approach combined with range-wide sampling to investigate species relationships and timing of speciation within North American *Haemorhous*.

2. Methods and materials

2.1. Taxon sampling and DNA sequencing

We sequenced 24 *Haemorhous* finches from across their respective distributions (Fig. 1, Table S1 in Appendix A). The populations of *H. mexicanus* east of the Great Plains region represent a recent human-mediated expansion (Wang et al., 2003; Hawley et al.,

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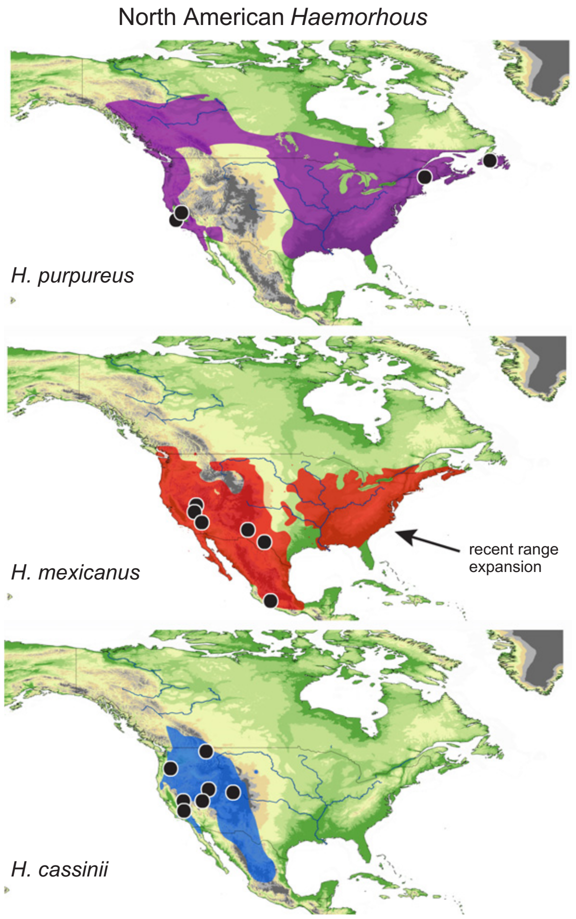

Fig. 1. Breeding distribution of North American *Haemorhous* finches, adapted from Ridgely et al. (2003). Dots indicate localities of samples used in this study.

2008), so we did not include samples from this region. We used *Carduelis pinus* as the outgroup (Zuccon et al., 2012).

Total genomic DNA was extracted from tissue using the DNeasy tissue extraction kit (Qiagen, Valenica, CA). We amplified one mitochondrial (NADH dehydrogenase subunit 2, ND2) and five nuclear markers (beta-fibrinogen intron 5, βFib5; eukaryotic translation elongation factor 2, EEF2; muscle-specific kinase receptor, Musk; ornithine decarboxylase, ODC; and rhodopsin, Rho1) using polymerase chain reaction (PCR) in 12.5 μl reactions using the following protocol: denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 2 min, followed by 10 min elongation at 72 °C and 4 °C soak. We adjusted annealing temperatures for each gene: βFib5 (60 °C), EEF2 (58 °C), Musk (58 °C), ND2 (54 °C), ODC (65 °C), and Rho1 (61 °C). Additional information on primers and the location of each locus are included in Table S2 in Appendix A. PCR products were sent to High-Throughput Genomics Unit (University of Washington, Seattle) for all subsequent sequencing steps. We aligned chromatograms of the forward and reverse strands in Sequencher 4.9 (GeneCodes Corporation, Ann Arbor, MI) and sequences were translated into amino acids to check for premature stop codons.

To resolve introns that had insertions/deletion events between homologous nuclear alleles, we used the program indelligent (Dmitriev and Rakitov, 2008). To phase heterozygous sites in the nuclear introns, we performed three separate runs for each marker in the program PHASE v.1.2 (Stephens et al., 2001). Haplotypes that had posterior probabilities of <0.90 were not included in subsequent analyses. We tested for recombination using the six different recombination tests in the program RDP3 (Martin et al., 2010).

2.2. Phylogeographic assessment

Prior to species tree reconstructions, which require *a priori* designation of species or populations, we broadly assessed phylogeographic structure within North American *Haemorhous* using MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001). We determined the best-fit sequence model based on Akiake Information Criterion (AIC) scores from Mr.Modeltest v.2.3 (Nylander, 2004). Analyses were run for 4 million generations, sampling from the posterior every 100 generations. We assessed MCMC convergence and determined burnin by examining ESS values and likelihood plots in the program Tracer v.1.5 (Rambaut and Drummond, 2010). We discarded the first 25% of trees as burnin.

2.3. Species tree reconstruction and divergence times

We reconstructed a species tree and estimated divergence times using *BEAST (Heled and Drummond, 2010), a part of the BEAST v.1.6.2 program (Drummond and Rambaut, 2007). The *BEAST method requires designation of species or populations prior to

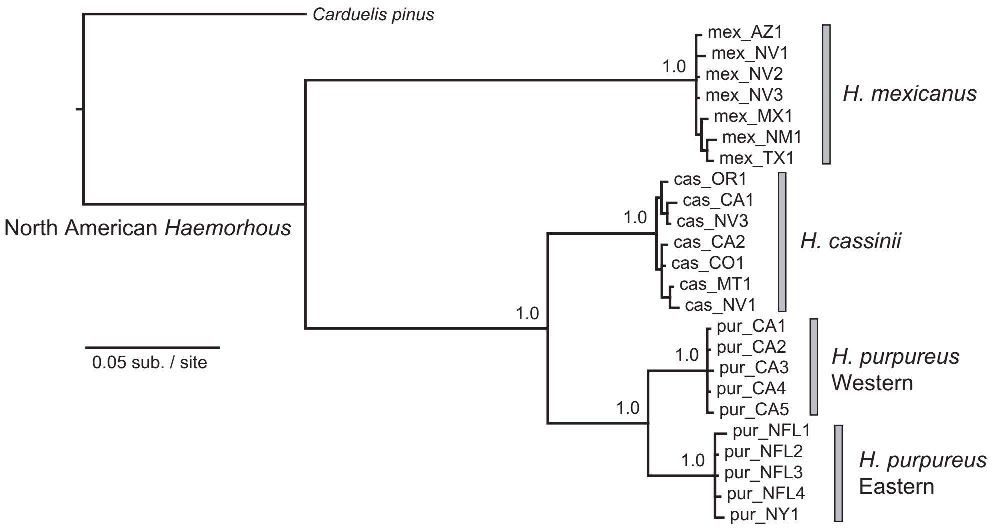

Fig. 2. Mitochondrial gene tree of North American *Haemorhous* finches based on mixed-model Bayesian inference of ND2 sequence data. Numbers at nodes indicate Bayesian posterior probability support values. Localities of haplotypes listed in Table S1 in Appendix A.