

Supplementary Text

Characteristics of the study species: Chickadees are a member of the tit family, *Paridae*. Of the seven species of chickadees inhabiting North America, the black-capped chickadee's range is the most expansive, extending from New Mexico in the south to Alaska and much of Canada in the north (Smith, 1991) (Figure 1A). The black-capped chickadee is a small (10-14g), non-migratory resident. This species lives in non-breeding flocks throughout the fall and winter before splitting into monogamous breeding pairs in early March for the spring and summer, typically laying eggs between May and June (Desrochers et al., 1988; Odum, 1942; Smith, 1991). Due to their broad distribution and local populations encompassing varying climactic conditions, these birds are expected to have a higher degree of metabolic and behavioral plasticity to cope with varying environments (Swanson et al., 2019).

The American goldfinch is a member of the finch family, *Fringillidae*, and is a small (11-20g) short-distant migrant. They are yearlong residents in Ohio and Massachusetts (our study locations), but they may engage in irregular migratory behaviors depending on food availability (Kaufman, 2023). They are a late-nesting species, with egg-laying not beginning until July (Holcomb, 1969). During their breeding season, they range as far north as Alberta and Quebec to as far south as Georgia in the United States (Figure 1B). However, in winter, they may be found as far south as Mexico. They also are polytypic, with four recognized subspecies (Gill et al., 2023a). This bird exhibits a high degree of sexual dimorphism, as well as phenotypic changes in their plumage coloration from vibrant yellow during their breeding season to olive in their non-breeding season (Kaufmann, 2023). So, similar to black-capped chickadees, they also exhibit a higher degree of phenotypic plasticity throughout their annual cycle.

Transcriptome annotation: The Trinotate v3.2.2 pipeline (Bryant et al., 2017) was used for the annotation of the transcriptomes. This pipeline includes the assignment of gene names, gene descriptions, and functional annotations to the assembled transcripts based on sequence similarity to known gene and protein databases. All the output files generated from the various annotation steps were loaded into Trinotate SQLite databases to generate annotation reports. Several transcripts identified as differentially expressed were not annotated by the Trinotate pipeline. For such cases, we manually extracted nucleotide sequences from the transcriptomes and queried them using the web-based BLAST v2.14.0 BLASTN tool against standard databases containing traditional GenBank and RefSeq RNA sequences ($e < 0.05$) to identify gene names and their associated functions.

Functional analysis of differentially expressed genes: PANTHER v18.0 database (Muruganujan et al., 2013) was used for gene family identification and protein class assignment for the list of differentially expressed genes. These gene lists were further fed into STRING v12.0, a web-based tool for the discovery of functional links between proteins (Szklarczyk et al., 2023). The gene lists were also fed into g:Profiler's for detection of significantly enriched (a) Gene Ontology terms (molecular function, cellular component, and biological process), (b) biological pathways (KEGG, Reactome, WikiPathways), (c) regulatory motifs (TRANSFAC, miRTarBase) and (d) protein complexes (CORUM, Human Protein Atlas) (Raudvere et al., 2019; Reimand et al., 2007). Heatmaps showing changes in expression among genes were created in R using the package pheatmap v1.0.12. The normalized expression data (TPM) was converted into a log-scale and the heatmaps were scaled by row for better visualization.

Aligning RNA-Seq reads to reference genomes and variant calling: We mapped the RNAseq reads to high-quality reference genomes of black-capped chickadee (*Poecile*

atricapillus) and the American goldfinch (*Spinus tristis*) (NCBI, 2023) using GATK-recommended best practices for variant discovery with RNA-Seq data (Caetano-Anolles, 2023; Van der Auwera & O'Connor, 2020). GATK HaplotypeCaller was used to call variants from the alignment file according to GATK's RNA-Seq short variant discovery recommendations (Caetano-Anolles, 2023, Poplin et al, 2017). After this step, the individual GVCF files were merged using the GATK CombineGVCFs tool, and GATK GenotypeGVCFs was used to perform joint genotyping on the multi-sample GVCF, generating a raw VCF file (Brouard, et al., 2019). The SNPs that were ultimately retained for further analyses were those with a minimum base quality of 20, a minor allele frequency > 0.01 , and present in all individuals (max missing count of 0). We further used a recently developed workflow from GATK (<https://github.com/gatk-workflows/gatk4-rnaseq-germline-snps-indels>), that is specifically optimized to call SNPs from RNAseq data to address challenges of calling SNPs from RNAseq data, arising from unequal expression of alleles.

Climate data extraction: The sampling locations' longitude and latitude coordinates were utilized in Daymet's Single Pixel Extraction Tool (Thornton et al., 2020) to retrieve minimum temperature, maximum temperature, and snow water equivalent for specified date ranges. This process was employed to calculate averages during cold seasons (November – March) across different years, as well as daily values for the month preceding sampling. Subsequently, the collected data was aggregated, and plots were created using the ggplot2 v3.4.2 package in R.