## FINAL PERFORMANCE REPORT



Federal Aid Grant No. F18AF00623 (T-106-R-1)
Winter Habitat Use and Seasonal Movements of Three Longspur Species

## Oklahoma Department of Wildlife Conservation

October 1, 2018 through March 31, 2023

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State: Oklahoma
Grant Number: F18AF00623 (T-106-R-1)
Grant Program: State Wildlife Grants
Grant Title: Winter Habitat Use and Seasonal Movements of Three Longspur Species
Grant Period: October 1, 2018 - March 31, 2023
Principal Investigator: Dr. Jeremy D. Ross, Oklahoma Biological Survey, University of Oklahoma

## EXECUTIVE SUMMARY:

During this 5-year project we focused on the wintering ecology and behavior of three focal longspur species [Chestnut-collared (CCLO), Smith's (SMLO), and Thick-billed (TBLO) (known formerly (at the beginning of this grant) as McCown's longspur)] as well as evolutionary patterns across the entire Calcariidae family. To accomplish this, we used a variety of field survey techniques, habitat, and vegetation analyses, traditional and automated radio-telemetry, and genomic analyses.

Our surveys indicated notable yearly variation in the abundance of each species at locations throughout the Southern Great Plains. This suggested that wintering longspur population sizes and distributions depend on perceptible habitat conditions at the time they arrive in late fall. Habitat analyses showed that longspurs differently use the landscape both in terms of their coarse-scale distributions corresponding to grassland biome types (SMLO $=$ tallgrass; CCLO $=$ mixed-grass; TBLO = shortgrass), as well as their fine-scale occupancy of plots based on vegetation and land cover characteristics [SMLO $=$ presence of Aristida grass and moderate vertical structure (presumably through grazing); $\mathrm{CCLO}=$ vertically and horizontally diverse structure (presumably through moderate-to-heavy grazing); TBLO $=$ short, highly disturbed grass (heavy grazing or arid conditions)].

Through the radio tracking of individual CCLO and, later, TBLO we discovered much about the facultative behaviors of longspurs in response to weather extremes, be they cold snaps or dry spells. Under extremely harsh, cold conditions most longspurs will move out of an area, either through dispersal or death, with only a minority of those returning to the area once the weather improves. Longspurs appear to often sample the upper air conditions in the early morning or late evening, and such behaviors often foreshadow a dispersal from the area or at least a reduction in local movement activities. During warm, dry conditions longspurs visit surface water sources earlier in the day, suggesting a need to promptly restore internal water balances.

We found that members of the Calcariidae family have been genetically divergent since the midPleistocene and differed in their population growth during the last ice ages. Some lineages showed evidence for selection within genes associated with thermal tolerance, which is unusual for such limited sampling within a taxonomic Family. For Snow Buntings (SNBU) and TBLO, in
particular, divergence of thermal tolerance genes may have been central to the causing or at least maintaining the split between these relatively closely-related species. The impacts of winter temperature extremes are probably most pronounced in TBLO \& CCLO at the northern extent of their wintering range and among SNBU and Lapland Longspur at their southern wintering extent (i.e., northern Oklahoma)

## BACKGROUND:

Grasslands are a highly threatened ecosystem in North America due to dramatic habitat loss, alteration and fragmentation leading to population declines in grassland birds that have been greater during the past fifty years than most other bird guilds (Vickery et al. 1999, Murphy 2003). Evidence is increasing that survival during the nonbreeding season has a strong influence on the population trends of migratory species (Calvert et al. 2009, Morrison et al. 2013).
Therefore, understanding winter survivorship of migratory grassland bird species may be central to their conservation, despite the paucity of winter ecology data for this guild.

Very few winter habitat studies have been conducted on obligate grassland-wintering birds, including the longspur species of conservation concern in the family Calcariidae. Many of the studies that have been completed are several decades old or were conducted in different systems (i.e., desert grasslands; Desmond 2005). Below is a synopsis of what is understood about the wintering ecology of Chestnut-collared Longspur (Calcarius ornatus; hereafter CCLO), Thickbilled Longspur (formerly called McCown's Longspur) (Rhynchophanes mccownii; hereafter TBLO), and Smith's Longspur (Calcarius pictus; hereafter SMLO):

- CCLO primarily winter in shortgrass prairie where vegetation is dominated by grasses and forbs $<0.5 \mathrm{~m}$ (Grzybowski 1982). Others have suggested that CCLO may use a wider range of habitats, such as taller grasses (Desmond et al. 2005). In Chihuahuan desert grasslands CCLO are positively associated with grass cover and grass height, but negatively associated with shrub cover (Desmond et al. 2005, Macías-Duarte et al. 2009).
- TBLO (formerly MCLO) winter in open habitats with sparse vegetation such as plowed fields, shortgrass prairie, and overgrazed pastures (With 2010). In West Texas TBLO was most abundant in lightly grazed pastures with vegetation height $<0.5 \mathrm{~m}$ (Grzybowski 1982). Smith (2004) found that playa wetlands managed for waterfowl attracted winter feeding flocks of TBLO.
- SMLO have been historically described as a species that inhabit large open prairies dominated by Aristida sp. during winter (Grzybowski 1982, Dunn and Dunn Jr. 1999, Holimon 2012). Studies have also suggested that other grasses and vegetation are also important such as Andropogon sp., Panicum sp., Sporobolus sp. and mosses (Grzybowski 1982, Dunn and Dunn 1999, Holimon et. al. 2012).

Though a solid framework of prior studies and first-hand expert knowledge exists regarding longspur wintering ecology, there remains some substantial research gaps. In Oklahoma and the entire Great Plains, there are fundamental unknowns concerning the habitat needs, mortality risks, flocking behavior, and seasonal movements of at-risk longspur species. This is of particular relevance in Oklahoma, as all three longspur species involved in this study are classified as Tier II species of greatest conservation need in the Oklahoma Comprehensive Wildlife Conservation Strategy (OCWCS 2015). Oklahoma Tier II designations are in line with
observed global trends for each of these species, with steep declines evident from Breeding Bird Surveys for CCLO and TBLO (data not available for SMLO) and for all three species from Christmas Bird Counts. Rapid declines in CCLO populations over the past 30 years were recently recognized by the International Union for the Conservation of Nature (IUCN) and BirdLife International when they changed the status of the species from Near Threatened to a Global status of Vulnerable (BirdLife International 2017). The rapid decline in CCLO increases the urgency to discover any factors that may be affecting the species on its wintering grounds (i.e., weather, predation, habitat fragmentation, or disturbance). These factors can be addressed by investigating winter site fidelity, seasonal movement, and seasonal social structure; all of which are known research priorities for the species (Bleho 2015).

Along with the need to better understand the winter ecology of each longspur species, there is also a need to study the grassland ecosystems they occupy and the wintering bird community to which they belong. Shortgrass Prairie, Mixed-grass Prairie, and Tallgrass Prairie are all listed as "Very High priority Conservation Landscapes" within their respective ecoregions (OCWCS 2015). In the face of downward population trends reported for each longspur species, there is an urgency to gather such fundamental ecological data during their overwintering life stage. Full life-cycle conservation has advanced in recent years by incorporating a broader diversity of biological subdisciplines, such as behavioral ecology. Although the usefulness of animal social network (e.g., flocking) analysis as a conservation tool is only in its infancy (Snijders et al. 2017) emerging knowledge such as how social structure in animal populations can change in relation to the environment has direct conservation implications. Social network analysis (SNA) is a promising technique to understand and predict population dynamics (Snijders et al. 2017) as it not only provides information on pairwise interactions between individuals, but also it can be used to examine how flocks collectively use the landscape depending on conditions (Hamede et al. 2009; Snijders et al. 2017). Subgrouping and home range delineation can be used to estimate critical habitat requirements and thresholds for occupancy in managed areas. Managers can also use the information gained through SNA to better understand how populations are socially integrated/differentiated and how this can affect efforts to survey and monitor species, especially more cryptic wintering populations of grassland birds. Spatial movement patterns in animals are closely linked with energetic requirements and the home range size of species may vary with habitat productivity and seasonal variation. As grassland birds are area-dependent for their preferred habitat (Vickery, Hunter, and Melvin 1994) we look to understand how wintering longspur home-ranges may shift in response to weather as a possible limiting factor for population occupancy of fragmented landscapes.

Genetic diversity is also a key contributor to conservation science. Through a genomic assessment of the longspur clade, we gained insights into how historical climatic fluctuations have been shaping population distributions through contractions and expansions throughout glacial and interglacial periods. We used genomic data to examine variation in known or suspected thermal tolerance genes across the latitudinal gradient of wintering distributions for the five species of Calcariidae that have historically wintered in Oklahoma. Through a wholegenome approach we looked for significant patterning among candidate genes for heat tolerance in CCLO, TBLO, SMLO, Lapland Longspurs, and Snow Buntings. Through a comparison of these data with shifts we modeled for each species' distribution from the Last Glacial Maximum to today, we suggest how extremes of winter cold versus heat might influence non-breeding
range, and whether this might point to winter range losses, including from Oklahoma, driving one or more species' declines.

## OBJECTIVES:

Objective 1: Estimate the geographic distribution and land use associations of SMLO, CCLO, and MCLO in Oklahoma using systematic transect and opportunistic sampling throughout the state's grassland regions. Summaries and maps will be provided in each report.

Objective 2: Develop species distribution models and conduct longspur-association analyses for all wintering bird species detected during our longspur surveys. Distribution maps and longspurassociation analyses for sufficiently-common species will be included in reports.

Objective 3: Build a multiscale habitat suitability model for each species from fine- and coarsescale vegetation structure and composition data gathered at a priori selected points along transects, as well as at observed longspur locations.

Objective 4: Determine daily/seasonal movement and winter site fidelity of CCLO through capture, banding, and radio telemetry tracking of individuals. Summaries and maps will be provided in each report.

Objective 5: Study historical drivers of speciation and wintering distributions for the longspur complex, look for genetic evidence of recent population expansions/declines as well as functional differences in thermal tolerance genes in this complex.

## APPROACH, RESULTS AND DISCUSSION BY OBJECTIVE:

Objective 1: Estimate the geographic distribution and land use associations of SMLO, CCLO, and MCLO in Oklahoma using systematic transect and opportunistic sampling throughout the state's grassland regions. Summaries and maps will be provided in each report.

## Transect Surveys

We used standard distance sampling techniques while walking along line transects to estimate the detectability and associated densities of each longspur species (and other suitably-common grassland bird species; see Objective 2) at various sites distributed throughout the study area (Figure 1). These sites comprised 16 private or public protected areas that spanned a gradient of grasslands and associated strongholds for wintering longspurs: from tallgrass prairie and Smith’s Longspur (SMLO; Calcarius pictus) in the east, to mixed-grass prairie and Chestnut-collared Longspur (CCLO; C. ornatus) in the center, to high plains shortgrass and Thick-billed Longspur (TBLO; Rhynchophanes mccownii) in the west.


Figure 1: Map of locations where walking-transect distance sampling occurred. Sites boundaries are highlighted as to whether they were surveyed only in 2018/19 ( $1^{\text {st }}$ Year; black), only in 2019/20 ( $2^{\text {nd }}$ year; yellow), or in both the $1^{\text {st }}$ and $2^{\text {nd }}$ years (blue). Note: areas surveyed in the $3^{\text {rd }}$ year (2020/21) were a subset of the areas previously surveyed and are included in the areas shaded blue.

To define transects we randomly mapped straight pathways onto each site that measured between $0.8-5 \mathrm{~km}$, depending on the size and shape of the protected area. We removed all transects that went through large portions of unsuitable habitat (open water, forest, bare rock, shrubland, etc.) as well as redundant transects (those very close to one another). As a result, transects were randomly distributed across the site in suitable habitats (see Figure 2). The number of transects per site depended on the amount of accessible area, with larger locations receiving more transects. The goal was to cover enough of the site to allow us to estimate both the distribution and density of the focal species. The exact number of transects per site can be found in Table 1. We limited habitat density calculations to those species that were detected at least 60 times during our surveys.

During the winter of 2018/19 we conducted 291 transects at nine different sites (Figure 1) ranging from 1 to 5 km in length, resulting in 370 km of survey effort. During these surveys we detected 67 species from our transects plus several more incidentally outside of our official surveys. Along our survey transects we recorded 1,951 flush detections (all species), 3047 total detection events (including incidentals and flyovers), and a total count of 14,805 birds. During the winter of 2019/20 we increased our survey coverage and conducted 364 transects at twelve different sites (Figure 1) ranging from 0.8 to 5 km in length, resulting in 494.4 km of survey effort (Table !! During that winter we detected 64 species along transects and, again,
incidentally observed several more species at these sites outside of the official surveys. Along our survey transects we recorded 1,972 flush detections (all species), 2,290 total detection events (including incidentals and flyovers), and a total count of 13,419 birds.


Figure 2: Map showing the distribution transects at Hackberry Flats Wildlife Management Area as an example of how transects were distributed within sites.

During the winter of 2020/21 we reduced our survey coverage due to the logistical challenges of the COVID pandemic but still covered 236 transects at six different sites, detecting 75 species despite covering less ground. Along our survey transects we recorded 1,194 flush detections (all species), 1,822 total detection events (including incidentals and flyovers), and a total count of 11,209 birds.

Table 1: Summary of where distance sampling was conducted and how much effort was spent. Each visit represents a replicate run of each transect.

|  | $2018 / 19$ |  | $2019 / 20$ |  | $2020 / 21$ |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Grassland Bird Survey Site | Visits | Transects | Visits | Transects | Visits | Transects |
| Tallgrass Prairie Preserve | 3 | 18 | 3 | 18 | 3 | 18 |
| Oka’Yanahli Preserve | 2 | 10 | 3 | 10 | 0 | 0 |
| Wichita Mountains Wildlife Refuge | 3 | 20 | 4 | 22 | 3 | 22 |
| Black Kettle National Grassland | 3 | 13 | 1 | 13 | 2 | 4 |
| Packsaddle WMA | 3 | 7 | 3 | 13 | 3 | 7 |
| Beaver River WMA | 3 | 6 | 2 | 6 | 0 | 0 |
| Optima NWR/WMA | 2 | 6 | 2 | 6 | 0 | 0 |
| Hackberry Flat WMA | 2 | 8 | 3 | 8 | 0 | 0 |
| Rita Blanca National Grassland | 3 | 17 | 3 | 17 | 3 | 17 |
| Cimarron Hills WMA | 0 | 0 | 2 | 2 | 0 | 0 |
| Cimarron Bluffs WMA | 0 | 0 | 2 | 3 | 0 | 0 |
| Kiowa NWR | 0 | 0 | 3 | 12 | 3 | 22 |
|  |  |  |  |  |  |  |

At each location where a longspur was detected, we recorded a GPS point location for later use in species distribution and habitat/vegetation association models. These data included individuals detected along transects as well as those detected opportunistically when driving between sites or when approaching or departing transect endpoints. Furthermore, we also visited multiple sites specifically seeking observations about longspur presence because the species were historically reported there or because satellite imagery indicated potentially suitable habitat. These locations included various locations in the Oklahoma panhandle, Foss Lake, Washita NWR, Cooper WMA, and around Westheimer Airport in Norman. To produce ecological niche models for each longspur species, we obtained environmental data from Chelsa/Bioclim and landcover data from the National Land Cover Database to create GIS layers (habitat, precipitation, canopy cover, etc.) Using the gathered species points and the environmental and land cover data layers, we used the program Maxent to model species distributions.

Shown on the following page are the resulting maps for the three focal species of longspur (Figure 3a-c) and a table indicating significant relationships (+ve or -ve) between land use and the probability of occurrence for several grassland species' (also summarized in Table 2).
a) CCLO

b) TBLO

c) SMLO

Figure 3. Maxent species distribution model for our three focal species of longspur: (a) Chestnutcollared Longspur (CCLO); (b) Thick-billed Longspur (TBLO); (c) Smith's Longspur (SMLO). The darker the area on the map, the higher the likelihood that the species will occur there in winter. Green dots indicate where presence locations were recorded.


From our observations combined with data sourced from eBird we were able to model wintering range maps for each longspur species (including Lapland Longspur). In Figure 4 you can see those overlapping ranges, as was produced for our publication (Muller and Ross 2022).


Figure 4. Wintering range of longspur species derived from eBird models of non-breeding distribution. Shading and first-letter abbreviations for each species delineate ranges of each species and areas of likely overlap (from Muller and Ross 2022).

Objective 2: Develop species distribution models and conduct longspur-association analyses for all wintering bird species detected during our longspur surveys. Distribution maps and longspur-association analyses for sufficiently-common species will be included in reports.

Our analyses scaled beyond longspur species to consider the distributions and habitat association of all grassland-obligate wintering birds that we detected in our study area in sufficient numbers. The approach was the same as used for the longspur species in Objective 1.

First, we were able to examine yearly and monthly variation in abundance for several species at different locations throughout the study area. The data revealed that species abundance often significantly differed between years and the direction of change (increase/decrease) depended on the species and, presumably, their wintering habitat needs. For example, CCLO became more abundant in the Wichita Mountains Wildlife Refuge (WMWR) from 2018/19 to 2020/21, but Savannah Sparrows (SAVS) abundance trended the opposite direction there during this period (Figure 5).


Figure 5: Estimated abundances of select species by year and location. LCSP = LeConte's Sparrow; see text for other abbreviations used. 95\% CI are shown.

Furthermore, on a monthly basis species showed changes in abundance that provided clues about how they use the landscape in winter. For instance, CCLO generally tended to decline in abundance from December through January and February (Figure 6). However, in the Rita Blanca National Grasslands (RB) this trend was violated for the species, presumably as early migrants were moving into this area in February.

For each species for which we had at least 60 detections we used Maxent to analyze the association between presence and various mapped datasets, such as landcover, climatology, and topography. From these models we could extract those factors that were significantly, positively, or negatively, with the probability of occupancy for each species. The results are presented in Table 2.

We likewise used Maxent to produce species distribution models for all other grassland birds for which we were able to gather a sufficient number of detections. Shown below are maps for some species variously associated with our three focal species of longspurs, such as the Loggerhead Shrike, Le Conte's Sparrow, and Lapland Longspur (Figure 7).


Figure 6: Estimated monthly abundances of species by year and location. HOLA $=$ Horned Lark; see text for other abbreviations used. 95\% CI shown.

Table 2. Relationship of Twenty Grassland Species to Model Variables. We designate relationships as being significantly positive $(+)$, significantly negative $(-)$, or where significant relationships were positive at small percentages but negative at large percentages ( -+ ). Variables include percent of landscape as grassland (\%Grass), mean daily diurnal temperature range (Range), annual precipitation (Annual Precip), variability between the wettest quarter and driest quarter (Precip Seasonality), mean daily temperature of the coldest quarter (Winter Temp), mean tree canopy cover (Tree Canopy), percent of landscape as "open" land cover type (Openness), and the difference in the highest and lowest elevation point (Topography). Species are listed using the 4 -letter alpha code used by the U.S. Bird Banding Laboratory (https://www.pwrc.usgs.gov/bbl/manual/speclist.cfm).

|  | \%Grass | Range | Annual Precip | Precip <br> Seasonality | Winter Temp | Tree Canopy | Openness | Topography |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| HOLA | + | + |  | + |  |  |  |  |
| NOFL | + |  |  |  |  | -+ |  |  |
| EAME | + |  | + |  |  | - |  |  |
| WEME | + | - |  | + |  |  |  |  |
| CCLO | + | + | - |  |  |  |  |  |
| SMLO | + |  | + |  |  |  |  |  |
| TBLO |  | + |  | + |  |  | + |  |
| LALO | + | + |  | + |  |  |  |  |
| AMKE | + |  |  |  |  | -+ |  | + |
| NOHA | + |  |  | + |  | -+ |  |  |
| LOSH | + |  |  |  | - | -+ |  |  |
| RTHA | + |  |  |  |  | -+ |  |  |
| SOSP | + |  | + |  |  |  |  | + |
| SAVS | + |  | - |  |  | - |  |  |
| VESP | + |  |  |  |  | -+ |  |  |
| LCSP | + |  | + |  |  |  |  |  |
| WCSP |  |  | - |  |  | -+ |  |  |
| FISP | + |  | - |  |  |  |  | + |
| ATSP | + |  |  |  |  | -+ |  | + |
| EABL | + |  |  |  |  |  |  | + |

a) LOSH (Lanius ludovicianus)

b) LALO (Calcarius lapponicus)

c) LESP (Ammodramus leconteii)

Figure 7. Maxent species distribution models for select other species: (a) Loggerhead Shrike (LOSH); (b) Lapland Longspur (LALO); (c) LeConte's Sparrow (LESP). The darker the area on the map, the higher the likelihood that the species will occur there in winter. Green dots indicate where presence locations were recorded.


Objective 3: Build a multiscale habitat suitability model for each species from fine- and coarsescale vegetation structure and composition data gathered at a priori selected points along transects, as well as at observed longspur locations.

The work associated with this section was published in the following peer-reviewed article: Muller, J.A. and Ross, J.D., 2022. Fine-scale habitat associations of Oklahoma's longspurs. The Journal of Wildlife Management, 86(6), p.e22258.

## Vegetation Analysis:

During each winter, we recorded vegetation data at three representative study locations:
Tallgrass Prairie Preserve (tallgrass prairie), Wichita Mountains NWR (mixed-grass prairie), and Rita Blanca National Grassland (shortgrass prairie). These vegetation points were associated with the flushing locations of 9 species (CCLO, MCLO (aka TBLO), SMLO, and six other grassland-obligate wintering birds) and two types of random points: (1) randomly-selected locations across the entire site and (2) locations 25 m from the bird location in a randomlyselected cardinal direction). At each point we measured minimum, maximum, and visualobstruction vegetation heights.

During the winter of 2018/19, we conducted a total of 583 vegetation plot analyses and in the winter of 2019/20, we conducted a total of 968 vegetation plot analyses. The mean visualobstruction heights for points associated with the flushing locations for CCLO, MCLO/TBLO, SMLO, Horned Lark (HOLA), Lapland Longspur (LALO), Western Meadowlark (WEME), Eastern Meadowlark (EAME), Savannah Sparrow (SAVS), and LeConte's Sparrow (LESP) are presented in Figure 8. Additionally, the means for other vegetation variables are presented in Table 3.


Figure 8: Mean vertical-obstruction vegetation heights associated with detections of each of nine select species of grassland-obligate wintering birds in Oklahoma.

We photographed and categorized vegetation composition within four $1 \mathrm{~m}^{2}$ plots that were located 10 m from the flushing location of the bird in each of the major cardinal directions (north, east, south, and west). These same protocols were used around random points. We used the program Samplepoint to overlay a 7x7 grid of regularly-spaced points on these photos and then for each point we scored the underlying ground cover as one of 12 types: grass, forb, bare, rock, litter, wood, shrub, (cow) patty, water, cactus, unknown, or other (see Figure 9 for example for CCLO at Rita Blanca NG). We analyzed 3,872 vegetation plot photos to determine the mean and variation in percent coverage for each of the various landcover types according to species. We also included any vegetation species that occurred at $>10 \%$ total cover for the roughly $12.5 \mathrm{~m}^{2}$ veg point.


Figure 92: Horizontal landcover composition of CCLO flush points at Rita Blanca NG

We evaluated the relative importance of multiple variables that may influence habitat selection. We used a classification tree base (binary recursive partitioning) to test habitat selection as a binary response of two groups, either occupied (flush points) and available but not occupied (random locations) (Figures 10-12). We also used classification trees to test possible habitat selection differences between two field sites (same species but two different locations). Classification trees are nonparametric tests that allow for powerful comparisons of categorical and continuous data simultaneously (De'ath \& Fabricius, 2000). Classification trees have the advantage of avoiding overfitting data and producing easily interpretable graphs. The classification tree used a Bonferroni adjustment to account for multiple comparisons when making splitting decisions. We included all variables to test for significant factors, including continuous variables (i.e., percent grass, percent forb, percent bare ground, percent rock, percent litter, percent woody, percent scat, percent cactus, number of shrubs within 10 m , obstructed grass height, maximum grass height, minimum grass height) and binary variables (i.e., presence of grazing activity, presence of prairie dogs, evidence of recent burning, year of study, and presence or absence of individual species of vegetation).

We also used a randomization test to determine which response variables were significantly different among two groups (i.e., flush or random point), the randomization test compared the difference in means of two observed (actual) groups of points (CCLO, TBLO, LALO, SMLO, Random or Constrained Random) to differences in means of two groups of randomized data (Table 3). The randomization test pooled the two groups of points and then randomly distributed the individual values into two new groups with sizes equal to the original two groups. Each mean of the two new groups was calculated and the difference between these two new means was determined. This randomization process was repeated 1000 times to form a test or null distribution that was then used to derive a P value to compare to the observed means. The randomization test assessed how likely it would be to obtain a difference in the observed means
as great or greater than the difference obtained if the data (point locations) were randomly distributed among the two groups being compared. We also used the randomization test to determine if there were differences in the response variables between locations. We also specifically examined how the nearby presence of Three-awn grasses (Aristida sp.) was predictive of each species flush points. See figure legends for more complete details.


Figure 10. Classification Trees for Smith's Longspur (SMLO) and Chestnut-collared Longspur (CCLO) at Wichita Mountains Wildlife Refuge. Panel A is between CCLO and adjacent-random, Panel B is between CCLO and CCLO roosting locations, panel C is between SMLO and site-random, panel D is between CCLO and site-random, and panel E is between SMLO and adjacent-random.


Figure 11. Classification Trees for Lapland Longspur (LALO), Chestnut-collared Longspur (CCLO), and Thickbilled Longspur (TBLO) at Rita Blanca National Grassland. Panel A is between CCLO and adjacent-random, panel $B$ is between CCLO and site-random, panel C is between LALO and site-random, panel D is between LALO and adjacent-random, panel E is between TBLO and site-random, and panel F is between TBLO and adjacent-random.


Figure 12. Classification Trees for Smith's Longspur (SMLO) at the Tallgrass Prairie Preserve. Panel A is for the comparison of SMLO and site-random, while Panel B is for SMLO and adjacent-random.

Table 3. Percentages for grazing, burned, prairie dogs, number of trees with 10 m , and distance to nearest tree, and the vertical vegetation structure for each species and each random point. An $R$ on the end of the species denotes "constrained" random point, and N on the end of the species denotes a "Night" point. Significant differences are denoted $b y a=$ difference from random, $b=$ difference from constrained, $c=$ difference from other location, $\mathrm{d}=$ difference from CCLO.

| $\mathbf{n}$ | Location | Species | Grazing | Prairie <br> Dogs | Burned | Trees | Tree <br> Distance | Obs | Max | Min |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 72 | TALL | SMLO | 95.8 ac | 0 c | 1.4 a | 0.014 a | 178.8 ac | 12.88 abc | 46.18 ab | 1.26 ab |
| 70 | TALL | SMLOR | 84.2 | 0 | 1.4 | 0.014 | 170.3 | 19.14 | 58.96 | 3.8 |
| 122 | TALL | Random | 27.87 | 0 | 11.4 | 0.28 | 124.7 | 26.86 | 71.14 | 11.78 |
| 94 | RB | CCLO | 41.5 c | 2.1 | 0 | 0.43 c | 168.2 c | 14.06 c | 39.32 a | 0.766 abc |
| 93 | RB | CCLOR | 31.2 | 6.4 | 0 | 0.48 | 167 | 14.6 | 37.8 | 1.18 |
| 36 | RB | TBLO | 88.9 a | 11.1 | 0 | 0 a | 217.3 a | 2.6 ab | 11.2 ab | 0.054 ab |
| 35 | RB | TBLOR | 88.6 | 8.6 | 0 | 0 | 211 | 10.4 | 24.82 | 1.64 |
| 79 | RB | LALO | 73.4 a | 16.4 a | 0 | 0.09 a | 191.6 a | 7.72 ab | 25 a | 0.44 ab |
| 78 | RB | LALOR | 75.6 | 7.7 | 0 | 0.06 | 190.2 | 9.86 | 28.2 | 1.088 |
| 113 | RB | Random | 50.4 | 3.5 | 1.77 | 1.2 | 154.9 | 12.84 | 36.32 | 2.38 |
| 128 | WMWR | CCLO | 70.3 ac | 10.3 a | 2.3 | 0.01 ac | 109.98 ac | 11.04 abc | 41.58 ab | 1.4 abc |
| 130 | WMWR | CCLOR | 68.46 | 9.2 | 2.3 | 0.04 | 105.5 | 14.16 | 49.5 | 2.8 |
| 59 | WMWR | CCLON | 13.6 ad | 3.4 | 0 | 0 a | 192.2 ad | 20.2 d | 52.2 d | 3.12 ad |
| 45 | WMWR | SMLO | 82.2 ac | 8.89 c | 0 | 0.02 a | 121.4 ac | 10.32 ac | 48.44 a | 1.732 a |
| 45 | WMWR | SMLOR | 75.5 | 8.89 | 0 | 0.02 | 120.6 | 11.12 | 50.48 | 2.6 |
| 109 | WMWR | Random | 37.6 | 1.8 | 0.9 | 3.5 | 45.65 | 17.98 | 57.8 | 4.84 |

Objective 4: Determine daily/seasonal movement and winter site fidelity of CCLO through capture, banding, and radio telemetry tracking of individuals. Summaries and maps will be provided in each report.

The work associated with this section was published in the following peer-reviewed article: Muller, J.A., Perera, N. and Ross, J.D., 2021. Winter space use and sex ratios of Chestnutcollared Longspurs (Calcarius ornatus) in Oklahoma. The Wilson Journal of Ornithology, 133(4), pp.589-600.

## Banding and Traditional Radio Telemetry

We focused our capture and marking efforts on CCLO at 3 flocking locations around the Wichita Mountains Wildlife Refuge: west of the Visitor's Center, the Holy City/Meers Junction (Figure 13), and the large Black-tailed Prairie Dog town along Highway 49 near the Refuge Headquarters. The marked birds near the Visitor's Center or Refuge Headquarters generally occurred as a single flock, whereas the birds at Holy City/Meers Junction were usually subdivided among 4-5 flocks spread out over the area. The Visitor's Center area usually hosted around 50-100 but on occasion the flock grew to 180 CCLO, which was the highest singlespecies count in the refuge during the winter.

During the winter of 2018/19, we captured and banded 55 Chestnut-collared Longspurs in the Wichita Mountains Wildlife Refuge. Of these, we fitted 44 with radio transmitters. Twenty-one (21) tracked individuals remained long enough in the area to obtain at least 15 separate days of positive detections. Of the remaining individuals, four lost their transmitters early (confirmed by recovery of working units), two were depredated, and 17 had unknown fates (left area soon after being tagged).

During the winter of 2019/20, we captured and banded 61 Chestnut-collared Longspurs in the Wichita Mountains. Of these, we fitted 46 with VHF radio transmitters. 27 tracked individuals remained long enough in the area to obtain at least 15 separate days of positive detections. Of the remaining individuals, five lost their transmitters early (confirmed by recovery of working units), two were depredated, and 17 had unknown fates (left the area soon after being tagged).


Figure 13: Minimum Convex Polygons (home range estimate) of CCLO captured at the Meers Junction and having at least 15 locations.

In both 2018/19 and 2019/20, we attempted during each day of the study period to record at least one location for each radio-tracked bird. We analyzed the location data for home range estimation by conducting Minimum Convex Polygon (MCP) (on those birds with at least 15 points; Figure 14) and using Kernel Density Estimation (KED) (on birds with at least 30 points), for both years.

In the Visitor's Center area of the Wichita Mountains, the mean MCP size of radio-tracked CCLO was 18 ha whereas at the Holy City/Meers Junction the mean MCP estimate was much higher at 77 ha . In the latter area, we also recorded the largest mixed-species flock of 250 CCLO + SMLO, although flocks there typically ranged from 100 to 150 . We also developed Kernel Density estimates of the $95 \%$ and $50 \%$ isopleths at a grid size of 30 m to produce more realistic area-use estimates (Figure 15). We used a least squares cross validation (LSCV) bandwidth estimator because other estimators are known to over-estimate home range size (Seaman and Powell 1996). This was necessary because some individuals moved between flocking locations and, thus, had artificially inflated MCP estimates, such as the 1726ha polygon encompassing the locations of individual CCLO20 (Figure 15).


Figure 14: Minimum Convex Polygon and Kernel Density Estimation (home range estimate) of a single CCLO’s daily location detections during its entire tracking period in winter 2018/19

The demographic makeup of wintering CCLOs captured in the Wichita Mountains was substantially skewed toward being male ( $\sim 79 \%$ ) and being older ( $\sim 70 \%$ of males were at least in their second winter; Table 4). Based on comparisons with sex- and age-ratio patterns reported for other continental migrant birds (Ketterson et al. 1979, Macdonald et al. 2015), the skewed ratio found in Oklahoma suggests that this region represents the northern portion of the species' wintering distribution. This underscores the likely importance of this area for driving carry-over effects among the more dominant, territorial males that would use the area as a geographic headstart to assist them in arriving on their breeding grounds earlier and securing the more desirable territories in the subsequent breeding season.


Figure 15: Individual "CCLO20" MCP estimation. This bird moved 4 times between the three main flocking locations: Refuge Headquarters (left), Visitor's Center (center), and Holy City/Meers Junction (right).

Table 4. Sex and age ratio (Second Winter or Older (SW+) versus. First Winter(FW)) of Chestnut-collared Longspurs captured at the Wichita Mountains Wildlife Refuge during the two winters of the study period. * denotes significant difference $\mathrm{p}<0.001$. We did not test for significant differences in age as we do not know what the expected ratio should be.

| CCLO <br> demographics | $2018-$ $2019-$ <br> 2019 2020 |  |
| :--- | :--- | :--- |
| $\mathrm{M} / \mathrm{F}$ | $42 / 13^{*}$ | $48 / 13^{*}$ |
| $\mathrm{SW}+/ \mathrm{FW}$ | $37 / 18$ | $43 / 18$ |
| n | 55 | 61 |

## Home Range Estimate Results

During the traditional radio-telemetry efforts we collected at least fifteen location points for each of 51 CCLO ( 24 in 2018/19 and 27 in 2019/20). Their minimum convex polygon (MCP) home ranges varied widely from 5.7 to $1,783.5$ ha, with a mean of 128.8 ha ( $\mathrm{SD}=291.9$ ). A linear regression of the number of points collected versus the MCP area did not indicate a significant relationship $\left(R^{2}=0.018 ; p=0.76\right)$. Among the 30 CCLO relocated at least thirty times the sexratio was male-biased ( $9 \mathrm{~F}: 21 \mathrm{M}$ ) and the age-ratio skewed toward older birds ( $6 \mathrm{FW}: 24 \mathrm{AFW}$ ).

The $95 \%$ KDE across all CCLO indicated a mean area of 29.87 ha (range $=1.15$ to 101.58 ; $\mathrm{SD}=$ 22.49) while the mean area of the "core" $50 \% \mathrm{KDE}$ was 5.3 ha (range $=0.02-20.73$; $\mathrm{SD}=4.31$; Figure 16). We found no significant differences in either MCP, $50 \%$ Kernal Density, or $95 \%$ Kernal Density between sexes or ages in either year or between years.


Figure 16: Minimum Convex Polygons (home range estimate), and Kernel Density Estimation of CCLOs captured at the WMWR and having at least 30 locations for a selected six CCLOs (adapted from Muller et al. 2021)

There was a relatively high degree of overlap among the home ranges of CCLO (Table 5), suggesting that these groupings were effectively clustering around resources and/or moving around the landscape as cohesive flocks.

Table 5. Mean percent overlap for individual longspurs at the three focal grassland tracts across both years of the study. The locations are Visitor center (VC), Meers Turnoff (MT) and Headquarters (HQ) and $n$ is the number of individuals at each site in each year with sufficient points for analysis.

| YEAR |  | LOCATION | $\begin{array}{l}\text { AVERAGE } \\ \text { OVERLAP }\end{array}$ | SD |
| :--- | :---: | :--- | :--- | :--- |$] \mathrm{n}$| VC |
| :--- |
| 1 |

## Automated Radio-telemetry

The other part of our effort toward this objective was focused on using an automated radiotelemetry system throughout the Rita Blanca and Kiowa National Grasslands of Oklahoma and Texas. To this end, we deployed receiver stations at least 9.5 km apart at 12 locations in that study area and refer to this as the Dustbowl Automated Radio-telemetry Network (DARN; Figure 17).


Figure 17: DARN receiver locations
Fortunately, the distance at which detections could be made far exceeded expectations and we amassed thousands of instances where signals from the transmitters fitted on longspurs were simultaneously detected by 3 or more receivers. This enabled us to develop theoretical expectations of signal strength relative to the distance and angle relative to each antenna's bearing as a means to better estimate the location of each bird any time it was detected. Figure 18 displays the theoretical coverage of our DARN antennas, based on calibration data derived from known locations of the birds.


Figure 18: Theoretical coverage of the DARN array. Hotter colors represent areas of higher detection probability. Axes are displayed in UTM (Zone: 13)

Our estimates of the positions of birds were relatively coarse-scale [mean (s.d.) error of 2.93 km ( 2.65 km ), although we expect that this accuracy could be improved with additional calibration of our analyses. The plot of all locations of all birds, as shown in Figure 19, indicates that most detections were made within the DARN, as should be expected provided that the data were drawn from instances where at least two towers were simultaneously "pinged".


Figure 19: Kernel density plot of all locations across all longspurs tracked within the DARN area during winters of $2020-21$ and 2021-22. The scale ranges from yellow $=1$ to purple $\sim 60$.

Beyond these coarse-scale positionings, the DARN data also allowed us to examine the timing of certain flight behaviors. First, if we consider the number of towers simultaneously pinged as a proxy for flight altitude, we found a highly significant bias toward the twilight period for highaltitude flights (Figure 20; $t=-11.06 ; p<0.0001$ ). This fits with field observations made by our team where longspurs would often climb nearly or completely out of sight near sunset.

Towers pinged by time to nearest twilight


Figure 20: Proximity of detection events to nearest twilight (sunrise or sunset) relative to the number of towers simultaneously pinged.

Furthermore, we also noticed that these high-altitude flights would often precede an exodus or at least a shift in movement behaviors the following day. Our analysis supported this conclusion, with pings detected across four or more stations followed by significantly fewer detections of the bird the following day (i.e., wherein detections separated by no breaks 30 s or less were considered the same "event"; Figure 21), which was significant either if we examined it as pooled data (ANOVA F-value $=32.02 ; \mathrm{p}<0.0001$ ) or with a linear regression ( $\mathrm{t}=-5.806 ; \mathrm{p}<$ 0.0001 ). This significant relationship was still highly significant if the cutoff from "few" to "many" towers was lowered from 4 to 3 . The relevance of the timing of these flights could be that the longspurs are sampling the upper atmosphere and/or visually scouting the landscape in anticipation of a probable dispersal event. This is another line of evidence that longspurs are likely facultative dispersers during the non-breeding period, evaluating local conditions and responding accordingly.

Our analyses (somewhat preliminary) of the relationship between weather conditions and the occurrence of radio-tracked individuals at a focal pond, as well as their dispersal from the study area, indicate that standing surface water in stock tank overflow pools may be particularly important for Chestnut-collared Longspurs (CCLO), especially after warm and dry overnight periods.


Figure 21: Relationship between the number of towers pinged during an event (few $=1-3$; many $>3$ ) and the number of times the bird was detected the subsequent day

Finally, the cold snap of February 10-17, 2021 revealed a sudden shift in CCLO occupancy and behaviors within the DARN area. Of 23 birds being tracked at that time six (26.1\%) moved southward (four later returned), six ( $26.1 \%$ ) disappeared with no evidence of departure (but no nighttime data are available from these solar-powered tags), and five ( $21.7 \%$ ) reduced their movement activity, while two ( $8.7 \%$ ) showed no perceptible change in behavior and four ( $17.4 \%$ ) weren't detected often enough before, during, and after to assess an effect. Therefore, of the 19 birds that we could evaluate for a shift, 17 (89.5\%) indicated some clear response to the cold snap, with 12 ( $63.2 \%$ ) leaving the area through dispersal or death.

In summary, Chestnut-collared Longspurs respond facultatively to severe weather events by departing, either temporarily or permanently, from the study area during cold snaps accompanied by snowfall. This longspur species appears to have large winter-season home ranges, which will affect the scale of optimal management for this declining bird.

Objective 5: Study historical drivers of speciation and wintering distributions for the longspur complex, look for genetic evidence of recent population expansions/declines as well as functional differences in thermal tolerance genes in this complex.

We were able to generate "short-read" compilations of complete genomes for 52 individuals across six Calcariidae species/subspecies. Each provided approximately 30x coverage of the genome; therefore, we have high confidence in the alignments that we have produced. With these data we examined the evolutionary history of this Family relative to past climate eras and putative niche distributions. Furthermore, we screened candidate genes related to heat tolerance for evidence of selection across this clade, such as heat shock proteins (HSPs).

## Historical Demographic Analysis of the Calcariidae Family

North American open habitats, such as the Arctic tundra and grasslands, have undergone cyclical transitions from glacial to interglacial periods since the mid-Pliocene. During glacial periods, the Arctic tundra experienced drastic geographic displacements at the edges of the ice sheets, while grasslands maintained relative continuity but at lower latitudes. Historical demographic analyses of Arctic birds verify the complex impact of this glacial-interglacial dynamic on the Arctic avifauna. In contrast, there are few studies on demographic responses to glacial pulses by grassland specialists. The Calcariidae family (longspurs and snow buntings) specializes in open habitats. It has boreal origins and diversifies into six species in three genera. In this study, we investigated how glacial cycles affected the demographic profile of five species, encompassing grassland specialists, Arctic tundra specialists, and mixed grassland and tundra users. We generated whole-genome sequences for each species to trace demographic history using PSMC (pairwise sequentially Markovian coalescent). Our results indicate that at the end of the last glacial period, grassland specialists presented the highest effective population sizes (Ne). This agrees with the fact that during the last glacial period, low temperatures, dryness, and low CO2 concentrations allowed its continuity south of the ice sheets. Pollen records and climatic reconstructions suggest a predominantly treeless landscape dominated by $\mathrm{C} 3 / \mathrm{C} 4$ grasslands, open shrublands, and scattered woodlands in the southern plains (Hall \& Valastro, 1995; Shao et al., 2018; Woillez et al., 2011).

The six family members are distributed in a north-south latitudinal arrangement. In this study we analyzed five species. We group them according to the predominant habitat they use. Calcarius ornatus and Rhynchophanes mccownii are medium-range migrants and grasslands specialists. Calcarius lapponicus and Calcarius pictus use the grasslands for wintering sites and migrate to the Arctic tundra in Canada and Alaska to breed. The Snow Buntings, Plectrophenax nivalis nivalis, and Plectrophenax nivalis tonwsendi are adapted to the arctic tundra.

Sampling - We obtained samples from the Sam Noble Oklahoma Museum of Natural History, the Museum of the North at the University of Alaska, and from blood samples collected by the Ross lab during winter fieldwork in Oklahoma / Texas.

Sequencing - The lab work (DNA extraction and library preparation) was performed at the Consolidated Core Laboratory at the University of Oklahoma. The sequencing process was done
at the Oklahoma Medical Research Foundation (OMRF). We performed whole genome resequencing in all the samples with a coverage of approximately 30 x .

Data analyses - We controlled the quality of the sequences with FastQC. To assemble the Binary Alignment Map (BAM) files, we used the Calcarius ornatus reference genome published by the B10K project. We obtained the BAM files using the Genome Analysis Toolkit (GATK) pipeline (Van der Auwera \& O'Connor, 2020).

Pairwise sequentially Markovian coalescent (PSMC) analysis uses information stored in a diploid genome to reconstruct demographic history in a time window of 10 Kya to 10 Mya . Each line in the graphic involves one whole genome sequence and represents the fluctuation in the
 followed the instructions in the PSMC GitHub manual (Li \& Durbin, 2011). We used the estimation of the mutation rate per generation $(\mu)$ for birds stated by Smeds et al (2016). We adjusted PSMC parameters using parameters in Nadachowska-Brzyska et al (2015) and constructed five PSMC per species. We chose five to have the same number of samples for each species (we have only five samples for the two Plectrophenax nivalis subspecies and Calcarius pictus). We performed an Admixture study to test gene flow among Calcariidae family species.

We constructed a phylogeny of the Calcariidae family from an assembly of 10 genes (two mitochondrial, one intron and seven exons), using the Thick-billed Euphonia (Euphonia laniirostris) and Rosy Thrush-Tanager (Rhodinocichla rosea) as outgroups. It should be noted that this is the first Calcariidae phylogeny to use nuclear genes to resolve problematic nodes in the relationship between C. lapponicus and its sister species, C. ornatus and C. pictus.

## Results

During the last million years, members of the Calcariidae family variably fluctuated with glacial cycles such that the Effective Population Size $\left(\mathrm{N}_{\mathrm{e}}\right)$ of different species suggested that some were more common than others at different times (Figure 22A). For example, our results indicated that Thick-billed Longspurs (Rhynchophanes mccownii) experienced a constant population growth since the penultimate glacial cycle and remained common through the end of the glacial period. Likewise, Chestnut-collared Longspurs (C. ornatus) retained large population sizes into the modern period. These grassland specialists seemed to benefit from conditions that prevailed through the late Pleistocene period, which suggested relatively abundant grasslands predominated throughout North America. By contrast, those species more typical of Arctic tundra (the Snow Bunting ( $P$. nivalis) subspecies) did not show evidence of explosive population growth, which would be consistent with that type of habitat existing as a relatively thin boundary between grassland-taiga and the glacial fields (Figure 22B).



Figure 22: Effective population size of each species/subspecies of the Calcariidae family estimated using Pairwise sequentially Markovian coalescent (PSMC) models of genomic DNA evolution. Highlighted in each panel are (A) time periods along the x -axis which correspond to major eras of the Pleistocene glacial period and (B) the general association of species along the $y$-axis according to their habitat associations throughout the year.

Our sampling afforded us the ability to contrast samples of two populations of Lapland Longspur (Calcarius lapponicus): C. l. alascensis which breeds west from the Mackenzie River in Alaska and the Bering Sea islands; C. l. subcalcaratus which breeds east of the Mackenzie River and winters in Oklahoma. The two populations showed different demographic histories, with those
sampled from island locations indicating much lower $\mathrm{N}_{\mathrm{e}}$ than those from interior sites (Figure 23). This suggests that populations breeding in western Alaska and on the Aleutian Islands have existed in isolation as smaller populations relative to Lapland Longspurs that migrate to Oklahoma in the winter.


Figure 23: $\mathrm{N}_{\mathrm{e}}$ of Lapland Longspur subspecies from west (upper panel) or east (lower) of the Mackenzie River. Note that "Sample36" was the only C.l. alascensis sampled from the interior of Alaska, suggesting that $\mathrm{N}_{\mathrm{e}}$ of island populations was reduced by biogeographic processes (isolation and area).

Variation within species was also evident in Snow Buntings, with similar patterns of reduced variation among non-migratory populations from Aleutian Islands (P.n. townsendii) relative to samples collected at mainland sites (P.n. nivalis; Figure 24).


Figure 24: $\mathrm{N}_{\mathrm{e}}$ of Snow Bunting subspecies from the non-migratory townsendii subspecies (upper panel) or the migratory nivalis subspecies. (lower). Note the very small population sizes in the former except "Sample43", which is from an island previously connected to Beringia during the Last Glacial Maximum, suggesting that $\mathrm{N}_{\mathrm{e}}$ of island populations was reduced by biogeographic processes (isolation and area).

An interesting observation from the Snow Bunting was that a purported non-migratory (P.n. townsendii) individual sampled from St. Paul Island indicated longer preservation of genetic population size. This can potentially be explained by this island's integration to the Bering land bridge during the Last Glacial Maximum (Figure 25) which underscores the evident information that can be gleaned from these PSMC analyses.


Figure 25: Map of the Beringia region showing the extent of the land bridge during the Last Glacial Maximum (yellow area, left panel) and the sampling locations of 4 of the 5 P.n. townsendii analyzed within the PSMC. Highlighted is "Sample43" from St. Paul Is, AK, which showed that larger populations remained in this area longer into the late stages of the glacial period.

There were further interesting patterns in the PSMC-estimated effective population sizes within species that exclusively winter in the southern Great Plains, the Chestnut-collared, Thick-Billed, and Smith's Longspurs. Most notable was the lack of variation in Smith's Longspur (C. pictus) relative to the other grassland obligates (Figure 26). This corresponds with this species' apparent lack of subspecific structure in contrast to the other Calcariidae examined. Furthermore, the Smith's Longspur's population growth appears to have been limited relative to the grassland specialists, with the former stabilizing during the middle of the Pleistocene while most populations of the other two species grew to astronomical sizes (note different scales in Figure 26) in the period following glacial retreat.


Figure 26: Variation within populations of PSMC-estimated effective population sizes for Smith's Longspur (upper), Chestnut-collared Longspur (middle), and Thick-billed Longspur (lower). Note the drastically different y-axis scales for each species.

With respect to the evolution of species with the Calcariidae, both admixture and phylogenetic analyses suggest deep divisions between all species except Chestnut-collared and Smith's Longspur. Our admixture analysis indicated that the most likely phylogenetic model was six independent ancestral populations (i.e., K6), with some support for five (K5). The K6 model differentiated all described species and even supported divergence between the two Snow Bunting subspecies (P.n. townsendii/nivalis) although some admixture was evident (Figure 27). Likewise, K5 supported strong divisions between Lapland Longspur, Thick-Billed Longspur, and Snow Bunting, but erased the boundary between Chestnut-collared and Smith's Longspur.

K6 (Six ancestral populations)


K5 (Five ancestral populations)


Figure 27: Cluster grouping from admixture analysis for the two top models of six (upper) and five (bottom) independent ancestral populations. Individuals are presented as columns and colored according to their probability of assignment to a cluster. Columns with multiple colors indicate an indeterminate assignment to each cluster represented.

Our multi-locus phylogeny of the Calcariidae family supported our admixture analysis, showing resolved but shallow divisions between Snow Bunting subspecies and the Chestnutcollared/Smith's Longspur complex (Figure 28). The topology of the tree further indicates relatively deep divisions between other branches of the tree, with both Lapland and Thick-billed Longspurs being relatively old lineages.


Figure 28: Phylogeny of the Calcariidae assembled across 10 coding and noncoding regions of the whole genome.

## Discussion

At the end of the last glacial period, the grassland specialists were the most abundant group in the Calcariidae family. During glacial periods grasslands expanded where aridity, low $\mathrm{CO}_{2}$ concentrations, and temperatures could not sustain forest-type habitats. Pollen records and climatic reconstructions suggest that sizable portions of North America at that time were predominantly treeless landscapes dominated by $\mathrm{C} 3 / \mathrm{C} 4$ grasslands, open shrublands, and scattered woodlands in the southern plains during the last glacial period.

Alarming numerical declines have been reported in grassland bird species. Fortunately, our results suggest that grasslands specialists in the Calcariidae family (Chestnut-collared and Thick-billed Longspurs) have entered this numerical decline with high levels of genetic variability. Yet, this does not entirely alleviate concern. In the case of the Carolina Parakeet (Conuropsis carolinensis), genetic variability analysis showed the species retained normal levels of heterozygosity near the time of extinction, indicating that the species decline was abrupt (Gelabert et al., 2020). The
species faced drastic Effective Population Size $\left(\mathrm{N}_{\mathrm{e}}\right)$ fluctuations caused by environmental factors that were exacerbated by human-induced pressures that ultimately drove the species to extinction.

Adaptative capacity under habitat condition changes during Anthropocene will be as crucial for open habitat specialists as it was during the glacial-interglacial transitions. At a molecular level, the additive genetic variance $\left(\mathrm{V}_{\mathrm{a}}\right)$ in critical traits will determine the adaptive capacity of species. Although it seems logical, the relationship between genetic variation ( $\pi$ ) and $\mathrm{V}_{\mathrm{a}}$ is poorly established. Studies suggest a stronger connection between $\mathrm{V}_{\mathrm{a}}, \mathrm{N}_{\mathrm{e}}$, and habitat connectivity (Kardos et al., 2021) which is particularly relevant in the context of our findings. For example, island populations of both Snow Bunting and Lapland Longspur indicated relatively low $\mathrm{N}_{\mathrm{e}}$. Provided the isolation and small geographic size of these populations, these relationships are not surprising. How this has affected these populations' ability to adapt to changing conditions is unknown, yet we do know that they are much more likely to be non-migratory. Since migration is a demanding event with many threats that individuals must respond to, the loss of genetic variation as a result of habitat fragmentation and population isolation could undermine these populations' capacity to adapt to varied and increasingly-altered conditions along the migratory pathway.

## Screening Candidate Thermal Tolerance Genes

Heat shock proteins (HSPs) are a large family of molecular chaperones that protect newly formed proteins while they fold and acquire their final three-dimensional structure. They also protect proteins during stressful stimuli, such as temperature extremes. Two widely studied genes, HSP70 and HSP90, play a critical role in cell survival under thermal stress. We have screened these genes within our Calcariidae genomes for evidence of (1) significant phylogenetic structuring and (2) evidence of significant non-neutral selection. For the first, HSP70, the phylogeny was poorly resolved (Figure 29a), potentially indicating that this gene is highly conserved within this Family. For the second, HSP90, our phylogeny indicated a well resolved topology, even from a small sample for each species/subspecies (Figure 29b).

Table 6: Results from McDonald-Kreitman tests of neutral selection among Calcariidae. Evidence of selection is highlighted in green (positive selection) or red (negative selection)

| Above diagonal: <br> Neutrality index (NI) <br> Below: proportion of <br> adaptive substitutions ( $\alpha)$ | Snow Bunting <br> (P. nivalis) | Thick-billed <br> Longspur <br> (R. mccownii) | Lapland <br> Longspur <br> (C. lapponicus) | Chestnut- <br> collared <br> Longspur <br> (C. ornatus) | Smith's <br> Longspur <br> (C. pictus) |
| ---: | :--- | :--- | :--- | :--- | :--- |
| Snow Bunting <br> (P. nivalis) | 0.787 | 2.919 | 1.833 | 1.079 |  |
| Thick-billed Longspur <br> (R. mccownii) | 0.212 |  | 0.000 | 0.000 | 0.000 |
| Lapland Longspur <br> (C. lapponicus) | -1.919 | -1.000 | 1.000 | 0.000 | 0.000 |
| Chestnut-collared Longspur <br> (C. ornatus) | -0.833 | 1.000 | 1.000 | 1.000 | 0.000 |
| Smith's Longspur <br> (C. pictus) | -0.079 |  |  |  |  |

Furthermore, for HSP90 we've conducted a McDonald-Kreitman test of neutral selection. This compares between two groups the degree of variation at synonymous coding sites versus nonsynonymous coding sites to determine if there has been any positive selection (overabundance of non-synonymous mutations that have altered the protein), negative selection (underabundance of such mutations), or neutral selection (random mutations across codons). In the pairwise
comparison of species, we found that the Snow Buntings showed evidence of positive selection relative to Thick-billed Longspurs, but negative selection relative to both Lapland and Chestnutcollared Longspurs (Table 6). Since positive selection indicates that the gene diverged faster than the species' neutral variation, HSP90 could serve very different roles for Snow Buntings and Thick-billed Longspur, which isn't surprising when considering that these two species occur at opposite ends of typical thermal niches (always cold/cool vs. always temperate/hot).


Figure 29: Maximum-likelihood trees across six species/subspecies of Calcariidae for heat shock proteins (a) HSPA2 [family A (Hsp70) member 2] and (b) HSP90B1 for heat shock protein 90kDa beta (Grp94), member 1

## RECOMMENDATIONS:

The following are some recommendations for future research related to the winter ecology of the three grassland-obligate longspurs and the management of their habitats.

1) Determine the relationship between Chestnut-collared and Smith's longspur occupancy and abundance under different grazing management strategies and/or prescribed burning frequencies. A comparison of occupancy and abundance could lead to grazing management recommendations for maintaining suitable wintering habitat for these species, both of which appear to occupy grazed sites primarily.
2) Invest in an expanded network of MOTUS towers that can detect the movement of tagged longspurs, and tag additional over-wintering birds at other sites. This can refine our understanding of arrival and departure dates for wintering longspurs, further document intraseason movements of wintering longspurs in relation to snowfall events, and strong cold fronts. As additional MOTUS towers are established across the Great Plains, these tagged birds could help to establish where the nesting grounds are located for the Chestnut-collared and Thick-billed longspurs that over-winter in Oklahoma.
3) Continue to monitor marked birds using MOTUS towers to evaluate their dependence upon water sources. The availability of water on a daily basis appears to be very important to Thickbilled and Chestnut-collared longspurs that over-winter in the Oklahoma panhandle, but this relationship has not been evaluated in the main body of the state.
4) The winter-season diets of Chestnut-collared and Thick-billed longspurs has not been determined. Based on the diets of other longspur species, it's almost certain that seeds comprise the majority of the winter diet for these two species, and a closer examination of the plant community at sites that are occupied by Chestnut-collared and Thick-billed longspurs may reveal finer-scale patterns of co-occurrence between one or both longspur species and specific plant species. This could lead to management recommendations that favor those plant species that are most likely to offer food and cover resources that are important for the over-winter survival of one or both of these longspurs.

## SIGNIFICANT DEVIATIONS: None

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