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Research Paper

Field research guided by citizen science and monitoring data reveal a novel alpine breeding distribution and vegetation associations of a declining, habitat-specialist songbird in Colorado, USA

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ABSTRACT. Documenting the breeding habitat and distribution of migratory songbirds is essential for accurately assessing their conservation status. The "sagebrush" subspecies of the Brewer's Sparrow (*Spizella breweri breweri*) breeds in greatest abundance in sagebrush-dominated (*Artemisia* spp.) shrublands of western North America, and the "timberline" subspecies (*S. b. taverneri*) breeds in shrubs or krummholz at or above the tree line in mountain ranges from Alaska to Montana. Brewer's Sparrows have also been reported at alpine sites in summer in mountain ranges across the western United States, but their taxonomic affiliation and breeding status are unknown. We reviewed monitoring, citizen science, and specimen data for Colorado and identified 186 historical summer observations of Brewer's Sparrows at 59 alpine sites (3334–4288 m elevation). We surveyed 39 alpine sites in June–July 2021–2023, detected a total of 100 adults (mostly singing males) at 26 alpine sites (3395–3754 m elevation), and confirmed breeding at three sites. Males occupied mixed-species willow patches 0.9–1.8 m tall, often intermixed with sparse conifer krummholz. We recorded songs and captured, measured, photographed, and collected blood and feather samples from birds at a subset of alpine sites and nearby, lower-elevation sagebrush sites in May–July 2021. Vegetation associations and timing of breeding at alpine sites closely matched those of *taverneri*, but short songs, external morphology, coloration, and genetics of alpine birds overlapped with sagebrush birds and more closely matched those of range-wide *breweri*. Our results indicate that western Colorado supports a widely-distributed, but poorly-documented, population of alpine *breweri* breeding in willows with sparse conifer krummholz within a relatively narrow elevation band at or above the tree line. Whether alpine birds are itinerant breeders that first nested in sagebrush remains unknown. Our results complicate interpretation of differences in breeding habitat and breeding phenology as supporting criteria for subspecific identification and taxonomic delineation in this species.

La recherche sur le terrain guidée par la science citoyenne et les données de suivi révèlent une nouvelle répartition de nidification en milieu alpin et les associations végétales d'un passereau spécialiste en baisse, au Colorado, États-Unis

RÉSUMÉ. Il est essentiel de caractériser l'habitat de nidification et la répartition des passereaux migrateurs pour évaluer avec précision leur situation. La sous-espèce « breweri » du Bruant de Brewer (*Spizella breweri breweri*) niche en plus grand nombre dans les régions arbustives dominées par les armoises (*Artemisia* sp.) de l'ouest de l'Amérique du Nord, et la sous-espèce « taverneri » (*S. b. taverneri*) niche dans les arbustes ou les krummholz à la limite des arbres ou au-dessus de celle-ci dans les chaînes de montagnes de l'Alaska au Montana. Des Bruants de Brewer ont également été signalés sur des sites alpins en été dans les chaînes de montagnes de l'ouest des États-Unis, mais leur appartenance taxonomique et leur statut de nidification sont inconnus. Nous avons examiné les données tirées de suivis, de science citoyenne et de spécimens pour le Colorado et identifié 186 observations historiques de Bruants de Brewer en été à 59 sites alpins (3334-4288 m d'altitude). Nous avons inventorié 39 sites alpins en juin-juillet 2021-2023, détecté 100 adultes (surtout des mâles chanteurs) à 26 sites alpins (3395-3754 m d'altitude) et confirmé la nidification à 3 sites. Les mâles occupaient des bosquets de saules d'espèces mixtes de 0,9 à 1,8 m de hauteur, souvent mélangés à des krummholz conifériens épars. Nous avons enregistré des chants et capturé, mesuré, photographié et prélevé des échantillons de sang et de plumes sur les oiseaux d'un sous-ensemble de sites alpins et de sites d'armoises à proximité et à plus basse altitude, en mai-juillet 2021. Les associations végétales et le moment de nidification aux sites alpins correspondaient étroitement à ceux de *taverneri*, mais les chants courts, la morphologie externe, la coloration et la génétique des oiseaux alpins chevauchaient ceux des oiseaux des armoises et correspondaient plus étroitement à ceux de *breweri* à la grandeur de l'aire. Nos résultats indiquent que l'ouest du Colorado abrite une population largement répartie, mais peu documentée, de *breweri* alpins nichant dans les saules avec krummholz conifériens épars, dans une bande d'altitude relativement étroite à la limite ou au-dessus de la limite des arbres. On ne sait pas si les oiseaux alpins sont des nicheurs nomades qui ont d'abord niché dans les armoises. Nos résultats compliquent l'interprétation des différences dans l'habitat de nidification et la phénologie de nidification en tant que critères usuels utilisés pour l'identification des sous-espèces et la délimitation taxonomique de cette espèce.

Key Words: *krummholz; migratory songbird; sagebrush; Spizella breweri; Spizella taverneri;subspecies; Timberline Sparrow; treeline*

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INTRODUCTION

Mapping the breeding distribution of declining, habitat-specialist songbirds is essential for accurately assessing their conservation status, and discovery of previously unknown breeding sites can improve understanding of vegetation associations, population size, and extirpation risk. The "sagebrush" subspecies of the Brewer's Sparrow (*Spizella breweri breweri*) is a migratory songbird that is widely considered a sagebrush-obligate because it breeds in greatest abundance in sagebrush (*Artemisia* spp.) shrublands across the contiguous western U.S. and southwestern Canada (Rotenberry et al. 2020). Trend estimates from the North American Breeding Bird Survey for *breweri* in sagebrush indicate a range-wide population decline of -1.01% per year from 1966-2015 (Sauer et al. 2017). In Colorado, *breweri* has been identified as a Tier 2 species of greatest conservation need in the State Wildlife Action Plan (Colorado Parks and Wildlife 2015) due to statewide population declines (-2.06%/yr) (Boyle and Reeder 2005) and historical and ongoing threats to their sagebrush breeding habitat. In contrast, the "timberline" subspecies (*S. b. taverneri*), or "Timberline Sparrow", typically breeds in shrubs and conifer krummholz (i.e., stunted, wind-deformed trees) at or above tree line in mountain ranges from east-central Alaska to northwestern Montana (Doyle 1997, Griffin et al. 2003, Rotenberry et al. 2020). Breeding *taverneri* were first found in northern British Columbia (Swarth and Brooks 1925), but later discoveries expanded the known breeding range of the subspecies south to southeastern Alberta and northwestern Montana (McTaggart-Cowan 1946, Semenchuk 1992, Griffin et al. 2003) and northeast to east-central Alaska (Doyle 1997).

Several authors have speculated that *taverneri* may also breed in mountain ranges farther south in the contiguous western U.S. based on summer records of singing male Brewer's Sparrows in shrubs or krummholz at or above tree line in California, Colorado, Idaho, Nevada, Oregon, Utah, and Wyoming (e.g., Lambeth 1998, Griffin et al. 2003, Hansley and Beauvais 2004). Colorado, in particular, has had numerous reports of Brewer's Sparrows in willow and conifer krummholz at alpine sites (i.e., those at or above tree line) in summer, but the taxonomic affiliation of such birds in Colorado and other western states remains unknown (Righter et al. 2004, Leukering 2008, Spencer 2014).

We identified several possible explanations for the summer occurrence of singing males in alpine areas (Table 1), each with different implications for Brewer's Sparrow breeding ecology and conservation status. First, alpine birds could be previously undocumented southern breeding populations of *taverneri* (Lambeth 1998, Righter et al. 2004). If so, this would extend the known breeding distribution of *taverneri* >1300 km south. Second, alpine birds could simply be "sagebrush" *breweri* breeding in an atypical habitat type (Andrews and Righter 1992, Righter et al. 2004). This subspecies occasionally nests in shrub communities other than sagebrush and at elevations up to ~3000 m (Righter et al. 2004, Walker et al. 2020, Zillig et al. 2023). Confirming *breweri* breeding in alpine areas would expand our understanding of the breeding biology and vegetation associations of this subspecies and potentially expand estimates of *breweri* population size. Under the second hypothesis, alpine populations of *breweri* might or might not be genetically distinct from those in sagebrush. If alpine *breweri* are distinct, it would suggest that sagebrush and alpine populations do not interbreed. If they are indistinguishable, it could suggest that sagebrush and alpine populations interbreed, that drift or selection has been insufficient for sagebrush and alpine populations to have diverged, or that alpine birds are itinerant breeders (i.e., *breweri* that first nest in sagebrush, then move upslope to renest in alpine areas; Spencer 2014). Third, alpine areas could represent a previously unknown contact zone between *breweri* and *taverneri*. The two subspecies are currently considered allopatric during the breeding season (Klicka et al. 2001, Mayr and Johnson 2001), but *taverneri* breeding range may extend farther south (Griffin et al. 2003). Fourth, alpine birds could be a third, previously undocumented subspecies. Existing genetic data only support the existence of two, rather than three taxa (Klicka et al. 1999), but Brewer's Sparrows in alpine areas south of *taverneri* breeding range have never been sampled. Finally, Brewer's Sparrows in alpine areas could also be non-breeding *breweri* or *taverneri*. However, an observation of recent fledglings being fed by adults at one alpine site in Colorado in early August (Lambeth 1998) makes non-breeding explanations less likely. Determining the taxonomic affiliation and breeding status of alpine birds would allow us to distinguish among most of these hypotheses.

Table 1. Hypotheses for the occurrence of Brewer's Sparrows (*Spizella breweri*) at high-elevation alpine sites in summer.

No.	Hypothesis
1	Alpine birds are *taverneri* breeding in typical habitat
2	Alpine birds are *breweri* breeding in atypical habitat
2.1	Alpine and sagebrush *breweri* form distinct genetic clusters
2.2	Alpine and sagebrush *breweri* are genetically indistinguishable
2.2.1	Alpine and sagebrush *breweri* interbreed
2.2.2	Alpine and sagebrush *breweri* do not interbreed but have not diverged
2.2.3	Alpine birds are itinerant breeders
3	Alpine birds are introgressed *breweri* x *taverneri* hybrids and backcrosses
4	Alpine birds are breeding populations of a third, previously unknown, subspecies
5	Alpine birds are non-breeding *breweri* or *taverneri* (e.g., post-breeding dispersers, summering transients, southbound migrants, or molt-migrants)

Determining their taxonomic affiliation may also help resolve ongoing taxonomic debate. The Timberline Sparrow was originally proposed as a new species, *Spizella taverneri* (Swarth and Brooks 1925), but it has always been considered a subspecies (AOU 1931, Chesser et al. 2022). The two taxa are estimated to have diverged within the past 35,000-80,000 years, so they are closely related (Klicka et al. 1999). Some authors argue that *taverneri* should be treated as a full species based on differences in genetics, morphology, song, vegetation associations, and timing of breeding (Klicka et al. 1999, Klicka et al. 2001, Mayr and Johnson 2001). If alpine birds in Colorado are breeding *taverneri*, this would raise the possibility that *taverneri* diverged from *breweri* via parapatric or peripatric speciation, then colonized alpine areas farther north, rather than diverging in allopatry at the northernmost end of *breweri* breeding range (Klicka et al. 1999). In contrast, if alpine birds are breeding *breweri*, it would counter arguments that differences in breeding habitat and timing of breeding necessarily support full species status for *taverneri* (Klicka et al. 1999, 2001).

The objectives of this study were to determine the taxonomic affiliation, breeding status, habitat characteristics, and summer distribution of Brewer's Sparrows in alpine areas of mountain ranges in western Colorado. To do this, we first compiled and reviewed historical summer records of Brewer's Sparrows to identify potential alpine breeding sites. We then surveyed a broadly distributed subset of alpine sites and lower-elevation sagebrush sites nearby. At each site, we surveyed for, counted, and recorded singing males, documented habitat features, looked for evidence of breeding, and captured birds to obtain morphological data, photographs, and blood and feather samples. Finally, we compared external morphology, acoustic structure of songs, plumage and maxilla color, and mitochondrial and nuclear DNA sequences between alpine birds and sagebrush *breweri* and against published data for each subspecies (Klicka et al. 1999, Walker 2024) to determine the taxonomic affiliation of alpine birds.

METHODS

Historical records

We compiled potential breeding-season (April-September) observations of Brewer's Sparrows in western Colorado from monitoring data from the Rocky Mountain Bird Observatory (1999-2005) and the Integrated Monitoring of Bird Conservation Regions program (2006-2020), a coordinated landbird monitoring effort administered by Bird Conservancy of the Rockies (Pavlacky et al. 2017), vetted eBird checklists from April-September (eBird 2020*a*, 2022), breeding bird survey data from the Boulder County Nature Association, field observations from U.S. Forest Service biologists, trip reports on birding list servers, and museum specimens from VertNet (www.vertnet.org). We summarized alpine records in June-July separately from those in August-September because the latter may represent post-breeding, dispersing, or migrating birds rather than breeding birds.

Site selection

We reviewed historical records in relation to elevation (above mean sea level), landcover, date, and extent of shrubs visible in imagery to identify potential sites to survey with the goal of maximizing our chances of locating breeding Brewer's Sparrows at each site. We first reviewed records in relation to a combination of elevation from a 10-m digital elevation model and landcover from a classified 25-m resolution vegetation layer (Colorado Vegetation Classification Project) to separate sites into high-elevation alpine shrubs or krummholz, subalpine shrubs, or lower-elevation sagebrush. We then restricted records to the first two-thirds of the breeding season (~June-July in alpine areas and ~April-June in sagebrush) to minimize inclusion of non-breeding records. We then examined the resulting records in relation to 1-m resolution, natural color imagery (National Agriculture Imagery Program) to qualitatively assess the extent of shrubs suitable for nesting. We then selected a non-random subset of alpine sites to survey based on a combination of elevation (> 3250 m), the number of historical breeding-season records, the extent of shrub-dominated landcover, land ownership (public only), ease of access (< 12 km from a road), and distance from other alpine sites to ensure broad spatial coverage. Finally, we selected a non-random subset of sagebrush sites to survey based on their proximity to selected alpine sites, elevation (1700-3100 m), number of historical breeding-season records, the extent of shrub-dominated landcover, ease of access, and land ownership.

Site descriptions

Sagebrush sites were on land owned by the Bureau of Land Management, the U.S. Forest Service, or Colorado Parks and Wildlife (CPW) with elevations ranging from 1746 to 3042 m. Most sagebrush sites were dominated by mountain big sagebrush (*A. tridentata vaseyana*), Wyoming big sagebrush (*A. t. wyomingensis*), or mountain silver sagebrush (*A. cana viscidula*). The San Luis Lakes State Wildlife Area site was dominated by black greasewood (*Sarcobates vermiculatus*) and the Tarryall State Wildlife Area site by shrubby cinquefoil (*Dasiphora fruticosa*). The highest elevation sagebrush sites, Land's End and Indian Point, had mountain silver sagebrush and mountain big sagebrush interspersed with shrubby cinquefoil and common juniper (*Juniperis communis*). Alpine sites were on U.S. Forest Service land, including several in designated Wilderness Areas, with elevations ranging from 3338 to 3764 m. Alpine sites were dominated by patches of willow (diamondleaf willow [*S. planifolia*], shortfruit willow [*S. brachycarpa*], and grayleaf willow [*S. glauca*]), or patches of willow mixed with sparse Engelmann spruce (*Picea engelmanni*) or subalpine fir (*Abies lasiocarpa*) krummholz or saplings. Willow patches varied in size but were typically surrounded by or interspersed with alpine tundra.

Surveys, recording, and breeding status

At each site, we searched for Brewer's Sparrows within patches of shrubs or conifer krummholz ≤ 3 m tall (Körner 1998). We visually tracked each singing male detected for up to 20 minutes and recorded songs with a Sennheiser® MKE600 shotgun microphone and Sound Devices® Mix-Pre 3 II digital sound recorder. We marked use locations (i.e., locations where males were singing or captured) using handheld GPS units (Garmin® GPSMap 64). If no birds were seen or heard during the first few minutes, we used brief song playback to elicit territorial responses. We recorded observations at alpine sites using eBird checklists and breeding codes (Confirmed, Probable, Possible) and evidence of breeding using behavior codes (eBird 2020*b*). We solicited assistance from experienced CPW and National Park Service volunteers and birders with surveying alpine sites as part of a citizen science effort. Volunteers and birders surveyed sites using similar protocols but did not record songs or capture birds.

Capture and sampling

Following observation and recording, we set up 3-m or 6-m Avinet® mist nets and used song playback with wireless speakers to attract and capture territorial, singing males. We banded each captured individual with a numbered, aluminum, size 0A, butt-end, U.S. Fish and Wildlife Service leg band. We confirmed birds were breeding-age (after hatch-year) by skull pneumaticization and wing and tail shape (Pyle 1997). We determined sex by the size and angle of the cloacal protuberance and extent of brood patch development. Only females are known to develop brood patches (Pyle 1997). We examined wing and tail feathers for evidence of active molt. We photographed males using a smartphone camera (Samsung Galaxy S7 or iPhone 11) in natural sunlight. Most images were stored in raw (DNG) format in each of three positions (front, back, and side) from ~25-35 cm away with a color standard card (X-Rite® ColorChecker Classic Mini). We plucked the two outermost tail feathers from each bird and stored feathers in glassine envelopes. We collected blood samples by puncturing the brachial-ulnar vein with a sterile, 26-gauge needle and collected the resulting droplet with a ~70µL non-heparinized capillary tube. We immediately transferred blood into a 1.5 mL centrifuge tube with Longmire's buffer. We closed and sealed each tube with Parafilm® prior to transport and storage. We kept feather and blood samples cool and shaded in the field, refrigerated them at ~1.7° C, then shipped them to the laboratory on ice. At the laboratory, we stored blood samples in a -70° C freezer (Owen 2011) and feather samples at room temperature.

Alpine habitat characteristics

Features of *breweri* breeding habitat in sagebrush are well studied (Rotenberry et al. 2020, Walker et al. 2020), so we only characterized habitat features at alpine sites. At each use location, we estimated the mean height of dominant shrub and tree species using 1.5 m tall mist net poles as a reference. We quantified landcover within a 100-m circular buffer around bird use locations and extracted elevation, slope, and aspect values from a 10-m digital elevation model in ArcPro® software, version 10.8.2 (ESRI, Redlands, CA). We first calculated the mean of each habitat variable across use locations for each individual, then used the mean for each individual to calculate summary statistics across individuals.

External morphology

We collected standard external morphometric measurements on males (Pyle 1997). We measured unflattened wing chord and tail length using an Avinet® stainless steel 15-cm wing/tail rule. We measured culmen length, bill width, bill depth, and tarsus length using Mitutoyo® stainless steel dial calipers. We measured culmen length from the distal edge of the nares to the tip of the bill. Bill width and bill depth were measured at the distal edge of the nares. With a sample size of males > 30, we anticipated statistical power > 0.971-0.999 to detect differences of 2.4-7.2% in morphological measurements based on means and standard deviations from *breweri* and *taverneri* measured in Montana (B. Walker, unpublished data). Only two observers collected morphological measurements, and both observers measured 35 of the same individuals. We used morphological measurements taken by the first observer (BLW) in analyses whenever they were available (n = 68). For birds only measured by the second observer (AAY; n = 14), we added the mean difference in measurements between the first and second observer to the second observer's values to account for potential inter-observer bias. The second observer's measurements differed from those of the first observer by an average of 0.38% (tarsus), -0.40% (tail), 0.61% (wing), 1.09% (culmen depth), 2.49% (culmen width), and -3.02% (culmen length). We measured mass using a 30-g Pesola® scale.

We compared external morphology between sagebrush and alpine males using two-sided t-tests with a sequential, ordered α adjustment for multiple tests (Benjamini and Hochberg 1995). We also compared values for alpine birds against morphological data from *breweri* and *taverneri* from range-wide studies (Klicka et al. 1999, Rotenberry et al. 2020). Male *taverneri* are larger on average, with longer wing, tail, and tarsus, larger mass (Klicka et al. 1999), and they reportedly have a narrower and shallower bill (Swarth and Brooks 1925, Doyle 1997, Klicka et al. 1999). We also used principal components analysis (PCA) to reduce the suite of seven morphological variables to fewer dimensions. We plotted the first two principal components and generated minimum convex hull polygons around songs from sagebrush and alpine sites to visualize and estimate overlap in overall acoustic structure. We also quantified the proportion of males that could be correctly classified to sagebrush or alpine by external morphology using random forest analysis (Cutler et al. 2007). We ran random forest analysis using the *rfPermute* function in the *rfPermute* package (version 2.5.1; Archer 2016) in R (version 4.1.3; R Core Team 2022). We included two random predictor variables (from the seven listed above) at each node split (*mTry* = 2), selected bootstrap samples with replacement, generated 5000 trees to ensure stable error estimates, and used a sample size equal to the number of males captured in each site type (n = 41 in sagebrush, n = 41 in alpine). We used random forest proximity plots to visualize the extent of overlap in external morphology between sagebrush and alpine males.

Song

Male Brewer's Sparrows sing two categories of song types, short songs and long songs, but each male typically gives only one short song type, rarely two or three (Walker 2000, Rich 2002). Males generally only give short songs when unpaired, so short songs are thought to play a key role in mate attraction (Walker 2000) and, therefore, in the potential for reproductive isolation between subspecies (Mayr and Johnson 2001). Acoustic elements of *taverneri* songs are reported to have lower maximum frequencies, higher minimum frequencies, and, therefore, cover a narrower range of frequencies than those of *breweri* (Klicka et al. 1999, Walker 2024).

We analyzed songs using Raven Pro® 1.6.3 (K. Lisa Yang Center for Bioacoustics 2023). We reviewed all recordings and selected one high-quality example of each short song type from each male for analysis. We added one recording from a public repository of a male singing short songs in willows in the Flattops Wilderness in Colorado to our alpine sample (XenoCanto 14188). Following Walker (2024), we used the selection tool to select (a) the entire song, (b) each section of the song, (c) one representative syllable type within each section, and (d) each note type within each selected syllable type. For each song, we measured song duration, total number of syllable types, and total number of note types. We then measured the following acoustic features on each selection: (a) peak frequency (the frequency at which maximum power occurred), (b) maximum frequency, (c) 95% frequency (the frequency that divides the selection into two intervals containing the lower 95% and the upper 5% of sound energy in the selection), (d) minimum frequency, (e) 5% frequency (the frequency that divides the selection into two intervals containing the lower 5% and the upper 95% of the sound energy in the selection), (f) 90% bandwidth (the difference between the 5% and 95% frequencies), and (e) aggregate entropy (the disorder in sound energy within the selection, a measure of sound complexity). We then calculated means for each variable across sections, across syllable types, and across note types for each song.

We tested for differences in acoustic features of short songs between sagebrush and alpine males using two-sided t-tests with α = 0.05 and with a sequential, ordered α adjustment for multiple tests (Benjamini and Hochberg 1995). We also compared the acoustic features of songs from sagebrush and alpine sites against those from *breweri* and *taverneri* songs across the species' breeding range (Walker 2024). We included ten acoustic variables that showed the largest difference between sagebrush and alpine sites in random forest analysis to identify their relative importance and significance in classification. We used the same random forest analysis parameters as those used in the analysis of morphological data (above), except that sample size for each site type in each run (n = 26) was one-half the smallest sample (alpine, n = 52) to avoid classification bias due to unequal sample sizes (Archer et al. 2017).

Most acoustic variables were moderately or strongly correlated, so we used PCA to reduce the suite of correlated acoustic variables to fewer dimensions. We included six variables identified by the random forest analysis as significant predictors in the PCA. We plotted the first two principal components and generated minimum convex hull polygons around songs from sagebrush and alpine sites to visualize and estimate overlap in overall acoustic structure.

Following Walker (2024), we also used song bandwidth and mean note bandwidth, two acoustic variables useful for separating short songs of *breweri* and *taverneri*, to estimate the probability that songs of sagebrush and alpine males were from *breweri*.

Plumage and maxilla color

We measured and compared the color of seven features reported to differ between *breweri* and *taverneri* between sagebrush and alpine males, including the top of the maxilla, base color of the back, breast, dorsal streaks, flanks, submoustachial stripe, and supercilium (Swarth and Brooks 1925, Pyle and Howell 1996, Doyle 1997), as well as the color of the eyestripe. Male *taverneri* were described as having the top of the maxilla dark brown to blackish and much darker than *breweri*. Male *taverneri* also have darker plumage overall, including grayish breast, flanks, submoustachial stripe, and supercilium, and a grayish-brown back with blackish dorsal streaks. In contrast, male *breweri* have a whitish breast, flanks, submoustachial stripe, and supercilium and a sandy-brown back with dark brown dorsal streaks.

We used the *micaToolbox* plugin (version 1.22; Troscianko and Stevens 2015) for ImageJ software (Schneider et al. 2012) to measure color in the visible spectrum (400-700 nm) from digital photographs. We converted raw images in DNG format to linear, normalized, grayscale red, green, and blue (RGB) reflectance stacks standardized against reflectance values for neutral gray 2 (3.22) and neutral gray 6.5 (38.40) standards to control for variation in ambient light. We calibrated each image using the 24-color standard card included in each photo to create a standardized, color-calibrated, multi-spectral image. We outlined a region of interest (ROI) for each feature and extracted mean linear, normalized mean RGB values across pixels within each ROI. This produced a dataset in which the color of each feature for each individual was represented as a point in three-dimensional linear, normalized RGB color space, with each point having three coordinates (RGB) scaled from 0-100. Points with coordinates closer to the origin (0, 0, 0; pure black) have lower reflectance on one or more axes.

We first checked for differences in color between sagebrush and alpine males by visually assessing whether the colors of each feature for each individual plotted in three-dimensional RGB color space clustered by site type and whether alpine males clustered closer to the origin as predicted if alpine birds are *taverneri*. We tested for statistical differences in the distributions of color between males at sagebrush and alpine sites using distance-based, non-parametric, permutational multivariate analysis of variance (PERMANOVA; Maia and White 2018). This procedure tests whether the centroids for points from sagebrush and alpine sites are in different locations in RGB color space, with the null hypothesis being that centroids are in the same location. Under the null, the observed distance between sagebrush and alpine centroids should be small and equivalent to distances obtained by permutation (i.e., repeated random reallocation of individuals to sagebrush or alpine). Distance-based PERMANOVA assumes that colors of each individual in the sample are independent and the dispersion of distances around the centroid within each site type are approximately equal. We ran PERMANOVA using the *adonis2* function in the R package *vegan* (Oksanen et al. 2022) with pairwise distances between points as the dependent variable and site type as the independent variable. If alpine birds are *taverneri*, the distance between centroids for sagebrush and alpine males should be greater than those obtained via permutation.

Genetics

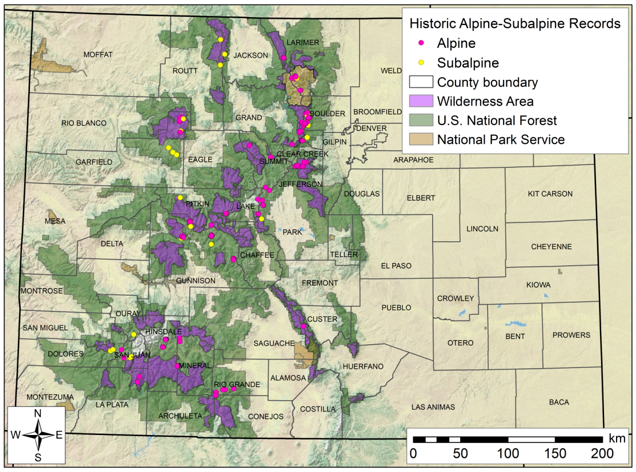
We extracted DNA from blood and residual tissue attached to feather quills and analyzed sequence data for the mtDNA cytochrome *b* gene following Klicka et al. (1999) (Appendix 1). Klicka et al. (1999) identified one base-pair substitution (locus 639) diagnostic for separating the subspecies. We then shipped DNA extractions on ice to the Bird Genoscape Project laboratory at Colorado State University and sequenced and analyzed the full genomes of 83 birds (Appendix 1). After identifying and removing three closely related individuals (kinship >0.0884), we calculated fixation indices for autosome (F_{ST}) and sex chromosome ($F_{ST\text{-}Z}$) scaffolds separately to test for genetic differentiation between sagebrush and alpine birds.

RESULTS

Historical records

We identified 186 June-July records of Brewer's Sparrows at 59 alpine sites with shrubs at or above tree line at elevations ranging from 3334 to 4288 m, as well as 37 records at 23 subalpine sites with shrubs below tree line at elevations ranging from 2929 to 3323 m, between 1914-2022 (Fig. 1; Appendix 1: Table S1). Of the 223 total records, 90 were from Rocky Mountain Bird Observatory/Bird Conservancy of the Rockies monitoring data (1999-2017), 86 from eBird (1995-2022), 33 from the Boulder County Nature Association's Indian Peaks Bird Count database, six from trip reports on birding list servers, four from U.S. Forest Service biologist field notes, two from VertNet specimen records, and two from The Colorado Breeding Bird Atlas (Lambeth 1998). Brewer's Sparrows were also reported in eBird in August-September (1999-2022) at 11 of those 59 alpine sites plus 30 additional alpine sites (Appendix 1: Fig. S1, Table S2).

Fig. 1. Historical records of Brewer's Sparrow (*Spizella breweri*) at alpine and subalpine sites in western Colorado from 1 June to 31 July, 1914–2022 (Appendix 1: Table 1).



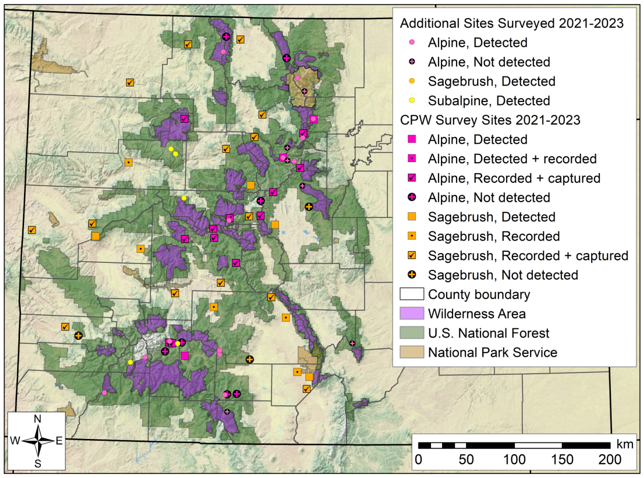
Surveys, capture, and banding

We conducted 57 surveys at 48 sites in May-July 2021-2023 (Fig. 2; Appendix 1: Table S3). We detected 181 males at 22 of 26 sagebrush sites surveyed and 78 males at 14 of 22 alpine sites surveyed (Appendix 1: Table S4). Alpine sites with detections ranged in elevation from 3395 to 3754 m. We captured and banded 41 males, one female, and one bird of unknown sex at 14 sagebrush sites and 41 males and one female at 12 alpine sites. No captured birds showed evidence of flight feather molt. Volunteers and birders reported 19 additional singing males, three adults of unknown sex, and four juveniles across 15 of 21 alpine sites they surveyed (including four sites also surveyed by CPW) (Appendix 1: Table S5). In combination, we detected total of 97 males, three adults of unknown sex, and four juveniles at 26 of 39 alpine sites surveyed. Combining our 2021-2023 survey results with historical records, Brewer's Sparrows have now been documented in June-July from at least 72 alpine sites in western Colorado. That number jumps to 102 sites if August-September records are included. Volunteers and birders also counted 16 males at five of five subalpine sites they surveyed (Appendix 1: Table S5).

Breeding status and timing

We confirmed breeding at three alpine sites (Appendix 1: Tables S4, S5). We captured a female with a nearly fully developed brood patch at Rollins Pass on 23 June 2022. Birders confirmed adults with dependent fledglings at Rollins Pass on 17 July 2022. We found an active nest with three eggs ~21 cm off the ground in a conifer sapling growing within a 44-cm tall willow at Guanella Pass on 23 July 2023. We found a dependent juvenile being fed by an adult at Hoosier Pass on 27 July 2023. We considered breeding probable at 10 other alpine sites (and one subalpine site) based on the number of territorial, singing males detected, the presence of suspected breeding pairs, observations of territorial defense, or males captured with fully developed cloacal protuberances.

Fig. 2. Results of Brewer's Sparrow (*Spizella breweri*) surveys at alpine and sagebrush sites by Colorado Parks and Wildlife (CPW) and at additional sagebrush, subalpine, and alpine sites by volunteers and birders in western Colorado in May-July, 2021–2023.



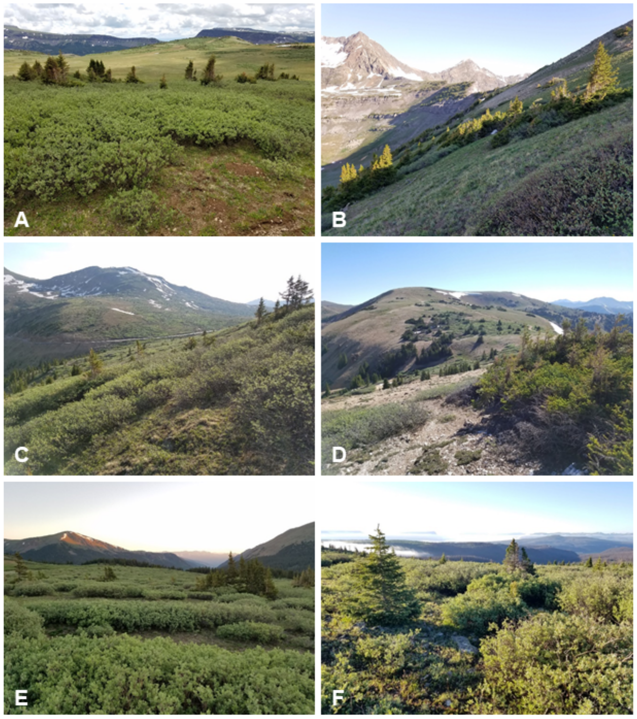
The absence of males at some alpine sites early in the season was informative about the timing of male arrival. Surveys at the Cumberland Pass, Scarp Ridge, Buck Mountain, and Kennebec Pass sites during 4-10 June detected only two males at the Buck Mountain site. At that time, willow leaves had just started to emerge and some willows were still buried under snow. In contrast, surveys from 15 June to 11 July detected seven males across those same four sites (Appendix 1: Tables S4, S5). Thus, males likely started arriving at alpine sites the second week of June (~June 8-10). Females likely first initiated nests during the last week of June. Based on an active nest with eggs on 23 July, nesting appears to continue through at least the end of July.

Alpine habitat characteristics

Use locations at alpine sites were typically in patches of willows, with or without conifer krummholz, growing in drier soil along the margins of larger, mesic willow patches or in isolated patches of willows surrounded by dry alpine tundra on ridges, slopes, or plateaus or in shallow basins (Fig. 3). At 172 use locations (n = 79 males) for which we recorded vegetation composition at alpine sites, 61.0% were in willow patches, 38.4% were in willow-dominated patches with sparse conifer krummholz cover, and 0.6% were in krummholz-dominated patches with sparse willows. At 136 use locations where we also collected vegetation height data, mean willow height averaged 1.3 m (range 0.9-1.8 m; n = 58 males) and mean conifer krummholz height averaged 2.6 m (range 1.3-5.0 m; n = 28 males). When conifer krummholz was present, males commonly sang from the tops of conifers above the surrounding willows.

Landcover within 100 m around use locations averaged 30.8% upland willow (range 0.0-100.0%), 3.9% conifer (range 0.0-67.3%), 56.3% alpine tundra (range 0.0-100.0%), and 9.0% bare ground/rock/snow (range 0.0-76.6%). Elevation at use

Fig. 3. Examples of typical vegetation structure, species composition, and topography at alpine sites where Brewer's Sparrows (*Spizella breweri*) were detected in western Colorado, including the following: (A) Devil's Causeway, (B) Scarp Ridge, (C) Independence Pass, (D) Taylor Pass, (E) Guanella Pass, and (F) Jarosa Mesa. Birds were typically found in patches of willows or willows mixed with sparse conifer krummholz in relatively dry soil on ridges, slopes, and plateaus (Appendix 1: Table S3).



locations at alpine sites ranged from 3458 to 3754 m (n = 172). Average slope at use locations had a median value of 18.5% (range 2.2-79.7%, n = 78 males). More males had use locations with a southern (136-225°), western (226-315°), or eastern (46-135°) aspect (33.3%, 28.2%, and 23.1%, respectively, of 78 males) than a northern (316-45°) aspect (15.4%).

External morphology

We collected morphological data on 82 males. We removed two outliers prior to analysis, including one sagebrush male with an abnormally long bill and an extreme value for culmen length (9.07 mm) and another sagebrush male that was abnormally heavy (14.7 g). We replaced each outlier with the mean for that variable across all other males in sagebrush in the PCA and random forest analyses.

Mean culmen length was 5.1% longer among alpine males, but there were no statistical differences in the six other metrics (Table 2). The distribution of culmen length values substantially overlapped between sagebrush and alpine males (Appendix 1, Fig. S2). Means for morphological metrics for both sagebrush and alpine males fell below (wing length) or within (tail length, tarsus length, and mass) the range of means reported for male *breweri* and, with the exception of tarsus, below the means reported for male *taverneri* by Klicka et al. (1999) and Rotenberry et al. (2020) (Table 2). Mean tarsus lengths for both sagebrush and alpine birds were similar to the mean reported for male *taverneri* in Rotenberry et al. (2020) but below the mean reported for male *taverneri* in Klicka et al. (1999).

The first two principal components explained 27.5% and 20.9%, respectively, of the variance in seven morphological variables in the PCA (Appendix 1: Tables S6, S7). Plotting the first two components indicated substantial overlap in external morphology between sagebrush and alpine males (Fig. 4A). Random forest analysis correctly classified 74.4% (95% CI: 63.6-83.4%) of 82 males to site type by external morphology, including 75.5% (95% CI: 59.7-83.4%) of sagebrush males and 73.2% (95% CI: 57.1-85.8%) of alpine males. Proximity plots also indicated substantial overlap in external morphology between sagebrush and alpine males (Fig. 4B).

Song

We analyzed 143 short song types from 134 males recorded at 19 sagebrush sites and 52 short song types from 49 males at 11 alpine sites (Appendix 1: Table S4). Five of 35 acoustic variables differed between sagebrush and alpine males (Table 3). Short songs of alpine males had narrower mean section, syllable, and note bandwidths, and higher mean note minimum and 5% frequencies than those of sagebrush males. The magnitude of differences between sagebrush and alpine males in those five metrics ranged from -11.8% to +5.3% (Table 3). Distributions of all acoustic variables from sagebrush and alpine sites showed substantial overlap (Appendix 1: Figs. S3-S6).

The first two principal components from PCA explained 55.4% and 15.2%, respectively, of the variance in ten acoustic variables included in the analysis (Appendix 1: Table S8). Bandwidth and maximum frequency variables loaded negatively on the first principal component, whereas mean minimum frequency and mean 5% frequency loaded positively on the second principal component (Appendix 1: Table S9). A plot of the first two principal components indicated that songs of alpine males were largely a subset of songs in sagebrush (Fig. 5A).

Random forest analysis indicated that three pairs of correlated variables were significant predictors of site type: mean note minimum and mean note 5% frequency, mean section bandwidth and mean section maximum frequency, and mean syllable bandwidth and mean syllable maximum frequency (Appendix 1: Fig. S7). Nonetheless, random forest analysis only correctly classified 67.2% (95% CI: 60.1-73.7%) of short songs to site type, including 65.7% (95% CI: 57.3-73.5%) of sagebrush songs and 71.2% (95% CI: 56.9-82.9%) of alpine songs. Proximity plots indicated that songs of alpine males were a subset of songs of sagebrush males (Fig. 5B).

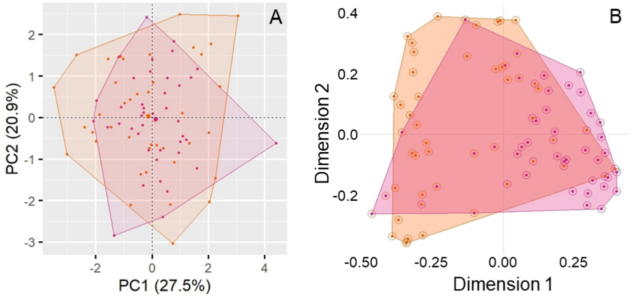
The PCA including range-wide data indicated that sagebrush and alpine short songs from Colorado more closely matched those of range-wide *breweri* than *taverneri* (Fig. 6). Based on song bandwidth and mean note bandwidth, 98.6% of 143 songs from sagebrush sites and 96.1% of 52 songs from alpine sites had a > 0.50 probability of being from *breweri*.

Table 2. Statistical comparison of external morphological variables for male Brewer's Sparrows (*Spizella breweri*) captured at sagebrush and alpine sites in western Colorado using a two-sample t-test with unequal variance. Values for linear measurements (mm) and mass (g) of sagebrush and alpine males are shown as mean ± SE. An asterisk (*) denotes variables that differed between sagebrush and alpine males after sequential Benjamini-Hochberg adjustment of α. Published values for *breweri* and *taverneri* are shown in the last four columns for comparison.

Variable	Sagebrush	n	Alpine	n	t	P	%	*breweri*[†]	*breweri*[‡]	*taverneri*[§]	*taverneri*[‡]
Wing length	61.39 ± 0.24	41	61.19 ± 0.21	41	0.61	0.54	-0.3	61.8-63.3	63.12 ± 0.17	64.1	64.67 ± 0.21
Tail length	60.67 ± 0.30	41	60.76 ± 0.27	41	-0.23	0.82	+0.2	59.7-64.3	62.33 ± 0.20	65.1	63.64 ± 0.43
Culmen length*	6.72 ± 0.05	40	7.06 ± 0.04	41	-5.56	<0.01	+5.1	No data	No data	No data	No data
Culmen width	4.01 ± 0.04	41	3.95 ± 0.03	41	1.15	0.25	-1.4	No data	No data	No data	No data
Culmen depth	4.33 ± 0.03	41	4.30 ± 0.02	41	0.84	0.40	-0.7	No data	No data	No data	No data
Tarsus length	17.42 ± 0.10	41	17.37 ± 0.07	41	0.48	0.64	-0.3	16.7-17.5	17.19 ± 0.08	17.3	17.84 ± 0.14
Mass	11.06 ± 0.09	40	10.88 ± 0.08	41	1.50	0.14	-1.6	8.9-11.8	11.06 ± 0.06	12.3	11.66 ± 0.10

[†] Values represent the range of means reported for male *breweri* in appendix 2 in Rotenberry et al. (2020).
[‡] Values represent mean ± SE reported for males of each subspecies in Klicka et al. (1999).
[§] Values represent the mean reported for male *taverneri* in appendix 2 in Rotenberry et al. (2020).

Fig. 4. Principal component plot (A) and random forest proximity plot (B) representing variation in overall external morphology for 82 male Brewer's Sparrows (*Spizella breweri*) in western Colorado, showing minimum convex polygons around males at alpine (pink points; n = 41) and sagebrush (orange points; n = 41) sites. Large points in (A) are the means for each site type. Circles around points in (B) show whether random forest analysis classified each bird as being from an alpine (pink circles) or a sagebrush (orange circles) site based on external morphology.



Plumage and maxilla color

We obtained images suitable for analysis from 36 males at sagebrush sites and 38 males at alpine sites (Appendix 1: Fig. S8). The distributions of RGB reflectance values substantially overlapped between sagebrush and alpine males for all features (Appendix 1: Fig. S9). Results of PERMANOVA indicated that alpine males had lower reflectance (i.e., darker colors) than sagebrush males for four of the eight features, including back ($F_{1,72}$ = 19.81, P < 0.01), breast ($F_{1,72}$ = 8.48, P < 0.01), eyestripe ($F_{1,72}$ = 5.62, P = 0.02), and flanks ($F_{1,72}$ = 20.31, P < 0.01), but not for dorsal streaks ($F_{1,72}$ = 0.68, P = 0.42), submoustachial stripe ($F_{1,72}$ = 1.45, P= 0.23), supercilium ($F_{1,72}$ = 2.25, P = 0.13), or maxilla ($F_{1,72}$ = 3.04, P = 0.08).

Genetics

We obtained feathers from all 85 birds captured. We obtained blood from 39 of 43 birds captured at sagebrush sites and 42 of 42 birds at alpine sites. We successfully extracted DNA and analyzed mtDNA from blood for 79 males and one bird of unknown sex (later confirmed as female), and from feathers from the remaining three males and two females. Sagebrush and alpine birds had similar mtDNA haplotype distributions. At sagebrush sites, 95.3% of 43 birds had a *breweri* haplotype and the remaining 4.7% had a *taverneri* haplotype (one male at Parlin and one male at Green Mountain Reservoir). At alpine sites, 95.2% of 42 birds had a *breweri* haplotype and 4.8% had a *taverneri* haplotype (one male at Rollins Pass and one male at Hoosier Pass).

We sequenced and analyzed the full genomes of 41 birds from 11 alpine sites and 42 birds from 13 sagebrush sites. After removing data from three closely related males (two from California Park and one from Land's End), we found 14,660,401 bi-allelic single nucleotide polymorphisms across the genomes of 80 individuals. Sagebrush and alpine birds showed no genomic differentiation in autosomes (F_{ST} = 0.000130, 95% CI: -0.0193-0.0485) or sex chromosomes (F_{ST-Z} = 0.000031, 95% CI: -0.0197-0.0481).

DISCUSSION

Three lines of evidence indicate that alpine Brewer's Sparrows found in western Colorado in June-July are *breweri* breeding in atypical habitat. First, despite similarities between alpine birds and *taverneri* in breeding phenology and habitat features and alpine males showing minor differences in song and plumage in the expected direction for *taverneri*, alpine males largely overlapped with sagebrush *breweri* males in most morphological and acoustic features and in coloration. Second, birds at sagebrush and alpine sites both had > 95% *breweri* mtDNA haplotypes and showed no genomic differentiation of either autosomes or sex chromosomes. Third, including an historical record of fledglings from the Flattops Wilderness on 3 August 1988 (Lambeth 1998), breeding has now been confirmed at four alpine sites in Colorado. Overall, our results point to three possibilities: (1) alpine *breweri* and sagebrush *breweri* interbreed,

Table 3. Statistical comparison of acoustic variables between 143 short songs from 134 male Brewer's Sparrows (*Spizella breweri*) at sagebrush sites and 52 short songs from 49 male Brewer's Sparrows at alpine sites in western Colorado with a two-sample t-test with unequal variance and a sequential, ordered α adjustment for multiple tests. Values are mean ± SE and % difference. An asterisk (*) denotes variables that differed between sagebrush and alpine sites. All frequency and bandwidth variables are in hertz (Hz; cycles/second).

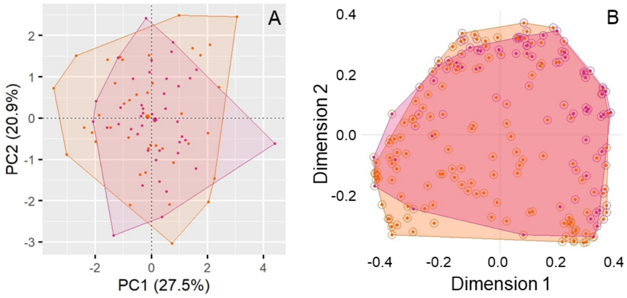
Variable	Sagebrush	Alpine	t	P	%
Song duration (seconds)	2.52 ± 0.04	2.69 ± 0.07	-2.14	0.03	+6.8
No. of sections/syllable types	2.29 ± 0.05	2.35 ± 0.08	-0.54	0.59	+2.3
No. of note types	4.20 ± 0.12	4.23 ± 0.19	-0.13	0.90	+0.7
Song peak frequency	4589 ± 64	4557 ± 112	0.25	0.80	-0.7
Song maximum frequency	9470 ± 97	9157 ± 132	1.91	0.06	-3.3
Song minimum frequency	2491 ± 76	2502 ± 45	-0.13	0.90	+0.5
Song bandwidth	7078 ± 97	6656 ± 129	2.60	0.01	-6.0
Song 95% frequency	5827 ± 66	5796 ± 102	0.26	0.80	-0.5
Song 5% frequency	3668 ± 36	3668 ± 49	0.00	1.00	0.0
Song 90% bandwidth	2159 ± 69	2128 ± 109	0.24	0.81	-1.4
Song aggregate entropy (bits)	4.37 ± 0.05	4.30 ± 0.07	0.71	0.48	-1.5
Mean section peak frequency	4652 ± 54	4625 ± 91	0.25	0.80	-0.6
Mean section maximum frequency	8642 ± 91	8327 ± 113	2.17	0.03	-3.6
Mean section minimum frequency	2634 ± 67	2678 ± 52	-0.52	0.60	+1.7
Mean section bandwidth*	6109 ± 88	5648 ± 114	3.20	< 0.01	-7.5
Mean section 95% frequency	5745 ± 59	5788 ± 97	-0.38	0.71	+0.7
Mean section 5% frequency	3718 ± 32	3779 ± 58	-0.92	0.36	+1.6
Mean section 90% bandwidth	2027 ± 56	2009 ± 94	0.17	0.87	-0.9
Mean section aggregate entropy (bits)	4.20 ± 0.04	4.08 ± 0.06	1.61	0.11	-2.9
Mean syllable peak frequency	4615 ± 52	4498 ± 97	1.06	0.29	-2.5
Mean syllable maximum frequency	8640 ± 91	8325 ± 113	2.17	0.03	-3.6
Mean syllable minimum frequency	2635 ± 67	2678 ± 52	-0.51	0.61	+1.7
Mean syllable bandwidth*	6108 ± 88	5647 ± 115	3.20	< 0.01	-7.6
Mean syllable 95% frequency	5764 ± 62	5829 ± 89	-0.60	0.55	+1.1
Mean syllable 5% frequency	3742 ± 32	3802 ± 60	-0.88	0.38	+1.6
Mean syllable 90% bandwidth	2021 ± 58	2027 ± 93	-0.05	0.96	+0.3
Mean syllable aggregate entropy (bits)	4.07 ± 0.04	3.94 ± 0.07	1.62	0.11	-3.2
Mean note peak frequency	5148 ± 50	5283 ± 64	-1.66	0.10	+2.6
Mean note maximum frequency	7508 ± 80	7379 ± 110	0.95	0.34	-1.7
Mean note minimum frequency*	3558 ± 61	3793 ± 52	-2.93	< 0.01	+6.6
Mean note bandwidth*	4065 ± 64	3586 ± 95	4.18	< 0.01	-11.8
Mean note 95% frequency	5869 ± 52	5991 ± 78	-1.31	0.19	+2.1
Mean note 5% frequency*	4528 ± 41	4766 ± 58	-3.34	< 0.01	+5.3
Mean note 90% bandwidth	1341 ± 27	1225 ± 44	2.25	0.03	-8.6
Mean note aggregate entropy (bits)	3.79 ± 0.03	3.70 ± 0.05	1.54	0.13	-2.4

(2) alpine *breweri* and sagebrush *breweri* do not interbreed but have not diverged, or (3) alpine birds are itinerant breeders. Although *breweri* regularly raise two or three broods in sagebrush (Mahony et al. 2002), documentation of itinerant breeding by North American songbirds is rare (Baldassarre et al. 2019).

We ruled out several other potential explanations. The lack of genomic differentiation between sagebrush *breweri* and alpine birds ruled out the possibility that alpine birds represent a distinct genetic cluster within *breweri*. Alpine areas are not a zone of introgressed *breweri-taverneri* hybrids or backcrosses because alpine birds were genetically indistinguishable from sagebrush *breweri* and fewer than 5% of alpine birds had *taverneri* haplotypes. There was also no geographic pattern in the occurrence of *taverneri* haplotypes as would be expected if Colorado contained a contact zone between subspecies. Alpine birds are not a third subspecies because we only detected *breweri* and *taverneri* mtDNA haplotypes. Although breeding has only been confirmed at four sites, as outlined previously, we suspect that all alpine birds we encountered in June-July were breeding birds. Although some birds in alpine areas conceivably could be dispersing, transient, or migrating *breweri*, all of the birds we captured were adults rather than juveniles and none showed evidence of prebasic flight feather molt typical of upslope post-breeding dispersers (Pyle et al. 2018).

Our results also indicate that Colorado supports a larger and more widely distributed breeding population of alpine *breweri* than currently recognized. First, despite limited surveys, we detected 100 adults at alpine sites statewide. This suggests Brewer's Sparrows are not uncommon in alpine areas, at least within specific vegetation associations and elevation ranges. Second, Brewer's Sparrows have now been documented in June-July at a total of 72 alpine sites across most major mountain ranges in the state. Farther north, *taverneri* often remain on alpine breeding territories through early September (Swarth 1936), so if birds reported at the 30 additional alpine sites in August-September also represent local breeders, then the breeding distribution of alpine *breweri* in Colorado is even broader. Third, birders unaffiliated with our citizen science effort reported Brewer's Sparrows at additional alpine sites in Colorado in 2023 (eBird 2023). Finally, there are undoubtedly many more alpine breeding sites in Colorado that have not yet been found considering the extent of unsurveyed willow and willow-krummholz patches at

Fig. 5. Principal component plot (A) and random forest proximity plot (B) representing variation in acoustic structure of short songs of Brewer's Sparrows (*Spizella breweri*) in western Colorado, showing minimum convex polygons around songs at alpine (pink points; n = 52 songs from 49 males) and sagebrush (orange points; n = 143 songs from 134 males) sites. Large points in (A) are means for each site type. Circles around points in (B) show whether random forest analysis classified each song as being from an alpine (pink) or a sagebrush (orange) site based on acoustic structure.



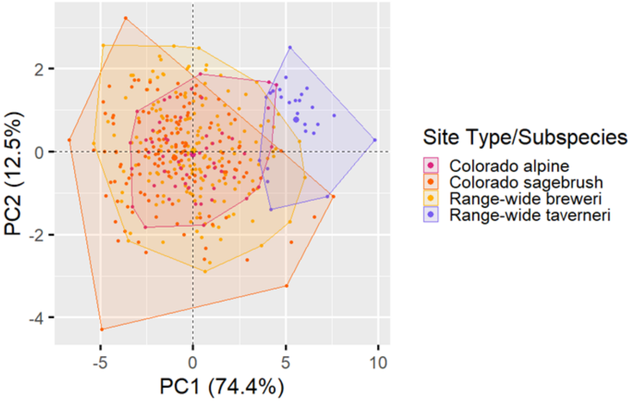
or above tree line statewide. Additional surveys and habitat modeling will be required to map their breeding distribution and estimate their abundance.

It remains unclear why the Brewer's Sparrow was not previously known as a widespread breeding species in alpine areas in Colorado. It may be because access to alpine areas is poor early in the breeding season when male song rates and detection probability are highest (Walker 2000) and birds have simply gone undetected in many alpine areas. Breeding *taverneri* were largely unknown prior to intensive, targeted surveys in east-central Alaska (Doyle 1997) and northwestern Montana (Griffin et al. 2003). Alternatively, Brewer's Sparrows may have only recently started colonizing alpine areas.

Notably, Brewer's Sparrows have also now been reported from 24 subalpine sites below tree line in June-July, including sites dominated by willows, shrubby cinquefoil, and snowberry (*Symphoricarpos* sp.). Confirmation of breeding at subalpine sites would further expand the known breeding distribution and vegetation associations of this subspecies. Although *breweri* is widely considered a sagebrush-obligate (e.g., Donnelly et al. 2017), our results support the conclusion that it is instead a shrub-obligate that nests in many different shrub species (Zillig et al. 2023).

Based on our results, we suspect that June-July records of territorial male Brewer's Sparrows in alpine shrub or krummholz communities in western states south of *taverneri* breeding range (e.g., California, Idaho, Nevada, Oregon, Utah, and Wyoming) are more likely breeding *breweri*, but additional surveys in British Columbia, Montana, and Washington are needed to confirm where the boundary occurs between breeding *breweri* and *taverneri*. Notably, singing and displaying male Sage Thrashers (*Oreoscoptes montanus*), another "sagebrush-obligate" species, have also been reported in willow stands at several alpine sites in Colorado (Righter et al. 2004, eBird 2023), including during our surveys, but whether they are breeding remains unknown.

Fig. 6. Principal component plot representing variation in acoustic structure of short songs of male Brewer's Sparrows (*Spizella breweri*), showing minimum convex polygons around songs of males at alpine (n = 52 songs) and sagebrush (n = 143 songs) sites in western Colorado versus songs of range-wide *taverneri* (n = 22 songs) and *breweri* (n = 178 songs) from Walker (2024). Large points are means for each site type or subspecies.



Similarities in vegetation associations and timing of breeding between alpine *breweri* in Colorado and *taverneri* farther north were striking. Both are generally restricted to shrubs or conifer krummholz within a narrow range of elevations above tree line (Doyle 1997, Griffin et al. 2003, Starzomski 2015, Stuyck et al. 2021). In Alaska, *taverneri* occur in large patches of 1.0-1.2 m tall alpine shrubs (diamondleaf, grayleaf, barrenground [*S. niphoclada*], Richardson's [*S. richardsonii*], and tealeaf willow [*S. pulchra*]) with an understory of stunted resin birch (*Betula glandulosa*), blueberry (*Vaccinium uliginosum*), and shrubby cinquefoil (Doyle 1997, Stuyck et al. 2021). In British Columbia and Alberta, *taverneri* breed in resin birch, bog birch (*B. pumila*), willow, and subalpine fir krummholz (Swarth 1930, Nordin et al. 1988, Doyle 1997). In Montana, *taverneri* are primarily found in subalpine fir krummholz sometimes with an understory of shrubby cinquefoil or common juniper (Griffin et al. 2003).

Although our survey approach precluded collecting comprehensive data on the timing of breeding at any given site, accumulated observations indicate that breeding phenology at alpine sites in Colorado was 5-7 weeks later than at sagebrush sites and closely matched that of *taverneri* farther north. Our data suggest that alpine males start arriving the second week of June and alpine females nest from the third week of June through the last week of July. Male *taverneri* start arriving as early as 29 May in British Columbia and Yukon Territory and mid-June in Alaska. Female *taverneri* are thought to initiate nests starting the third week of June, with fledging starting the second week of July (Swarth 1930, Swarth 1936, Doyle 1997, Stuyck et al. 2021). In contrast, male *breweri* first start arriving in mid-elevation sagebrush sites in western Colorado in mid-April and initiate nests by mid-May (Lambeth 1998, Righter et al. 2004, Magee 2016, eBird 2022). Alpine snow melted early in Colorado in 2021 due to below-average snowpack and minimal May-June precipitation, so the timing of arrival and breeding at alpine sites in Colorado may be even later in years with normal or above-average snowpack. Determining whether alpine Brewer's Sparrows are itinerant breeders is a priority for future research.

Differences in breeding phenology and habitat characteristics between populations are widely thought to contribute to reproductive isolation and may ultimately lead to speciation. Sagebrush and alpine *breweri* breed in close proximity in Colorado (< 12 km), but timing of breeding and habitat features used by alpine birds more closely match those of *taverneri* > 1300 km away. Our findings complicate interpretation of differences in timing of breeding and breeding habitat as supporting criteria for subspecific identification and taxonomic delineation in this species (contra Klicka et al. 1999, 2001). The proximity of sagebrush and alpine breeding sites in Colorado may facilitate interbreeding (or itinerant breeding), which in turn, would prevent reproductive isolation and subsequent genetic divergence between sagebrush and alpine populations.

Some plumage and song features of alpine birds showed tendencies toward *taverneri*. However, differences in plumage color between *breweri* and *taverneri* have never been quantified, so it is unclear if the differences in plumage we found are taxonomically relevant or whether alpine birds represent an intermediate phenotype between subspecies. Future studies of plumage color in the Brewer's Sparrow should measure the full spectrum of light visible to songbirds (~300-700 nm). We also lacked species-specific data on the spectral sensitivity of photoreceptors, so were unable to convert reflectance to cone-catch images that more closely represent what birds see. For that reason, we were unable to confirm that the color differences we measured were perceptible to other males and females (Maia and White 2018). Alpine males also gave short songs with narrower mean frequency bandwidths, which is in the expected direction if alpine males were *taverneri*, but the absolute magnitudes of differences we observed (5.3-11.8%) were much smaller than those between subspecies (36.7-42.6%, Walker 2024). Nonetheless, the cause of narrower frequency bandwidths among alpine males would be worth investigating in light of their relevance to reproductive isolation and taxonomy. In the absence of genetic differences, environmental influences associated with high-elevation environments could produce similarities in plumage and song between alpine *breweri* and *taverneri* farther north.

Our study highlights the value of combining data from formal monitoring programs, citizen science efforts, and species-specific field research to document the distribution of bird species that breed in remote, mountainous regions. Most state and federal conservation assessments for the Brewer's Sparrow in the western U.S. typically only consider sagebrush shrublands as *breweri* breeding habitat (e.g., Hansley and Beauvais 2004, Boyle and Reeder 2005). Our results will need to be incorporated into updated state and federal conservation assessments and breeding bird atlas accounts for *breweri* in Colorado and other western states to reflect the alpine (and possibly subalpine) breeding distribution and vegetation associations of this subspecies.

Author Contributions:

Conceived the research idea, study design, and methods: BLW. Conducted field surveys and sample collection: BLW and AAY. Processed genetic samples, analyzed genetic data, and wrote the genetics methods sections: CLB and CMB. Processed sound recordings and digital images, analyzed morphometric, acoustic, and color reflectance data: BLW. Wrote the paper: BLW. Reviewed and substantially edited the paper: BLW, AAY, CMB, and AWJ.

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APPENDIX. Supplemental materials for Walker et al. (2024), “Field research guided by citizen science and monitoring data reveal a novel alpine breeding distribution and vegetation associations of a declining, habitat-specialist songbird in Colorado, USA”

METHODS

Mitochondrial DNA

We isolated DNA from blood using standard protocols for Qiagen DNeasy Blood & Tissue Kits (Qiagen Inc., Valencia, California, USA). We isolated DNA from feathers using a Qiagen DNeasy Blood & Tissue Kit with 20 µL of DTT (dithiothreitol) added to the initial digestion step and an extended 48-hour digestion period with an additional 20 ul of protenase K added on the second day.

We amplified the mtDNA cytochrome *b* using the primers L14841 (AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA) (Kocher et al. 1989) and H4a (AAG TGG TAA GTC TTC AGT CTT TGG TTT ACA AGA CC) (Harshman 1996). We carried out polymerase chain reaction (PCR) for amplifying cytochrome *b* with an initial denaturation period of 1 min at 95° C followed by 30 cycles of denaturation for 15 secs at 95° C, primer annealing for 15 secs at 56° C, and polymer extension for 30 secs at 72° C followed by a final extension period of 10 min at 72° C. We cleaned PCR products using ExoSAP-IT (Affymetrix, Inc.) and sequenced them at the Case Western Reserve University Genomics Core facility (Cleveland, Ohio, USA) with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) using the same primer pair. We edited and aligned sequences using Geneious (Biomatters Limited).

Genomic DNA

We prepared whole genome sequencing libraries following Illumina’s Nextra Library Preparation protocol with minor modifications (Schweizer and DeSaix 2023). We sequenced pooled libraries on one NovoSeq 6000 lane at Novogene Corporation, Inc. with a target sequencing depth of 6x per individual. We trimmed the sequence data to remove potential PCR artifacts using the program Trimmomatic version 0.39 (Bolger et al. 2014), removing Illumina adapter sequences and removing polyG tail using a sliding window approach. We mapped reads to a reference genome of the closely related White-throated sparrow (*Zonotrichia albicollis*; GCF_000385455.1) using Burrows-Wheeler Aligner software version 0.7.17 (Li and Durbin 2009). After mapping, we sorted and converted the resulting SAM files to BAM files using SAMtools version1.16 (Li et al. 2009). We added read groups using *picard* version 3.0.0 (Broad Institute 2019) and marked PCR duplicates with SAMtools version 1.16 (Li et al. 2009). We calculated sequencing depth using the *genomecov* function of BEDtools (Quinlan and Hall 2010). We summarized mean and range of depth of coverage of individuals for each sampling site to determine if there was sequencing bias among sites. Initial population genetics analyses revealed an effect of variation in sequencing depth among individuals (range 4.7 – 6.8). To reduce sequencing depth variation, we followed the recommendations of Lou and Therkildsen (2022) and downsampled BAM files to 5x coverage using *picard* DownsampleSam (Broad Institute 2019).

We used all downsampled BAM files to create VCF files with the HaplotypeCaller function of GATK version 3.7 (McKenna et al. 2010, Van der Auwera et al. 2013). To facilitate faster computation time, we processed BAM files in approximately 3 Mbp genomic intervals and combined the resulting VCF files with the GatherVCFs function in GATK. This provided a single VCF file with a master set of genomic sites to be filtered appropriately for subsequent analyses. All indexing of VCF files was performed with the index function in BCFtools version 1.16 (Li 2011). For statistsical analyses, we extracted high-quality variants from the master VCF file by filtering for biallelic sites (-m 2 -M 2) with a minor allele frequency of at least 0.05 (--min-af 0.05, --max-af 0.95), a sequencing quality score of at least 30 (‘QUAL > 30’), and that were missing from less than 20% of the individuals sampled ('F_MISSING < 0.20') using the view function of BCFtools (Li 2011).

Given that signatures of population structure can be skewed by closely related individuals, we used NGSrelate version 2 (Korneliussen and Moltke 2015; Hanghøj et al. 2019) to identify and remove individuals with up to second-degree relationships (kinship >0.0884). A total of 3 birds were removed from the analysis. We calculated genetic differentiation between Brewer’s Sparrows from sagebrush and alpine sites in vcftools (Danecek et al. 2011). Given potential variation in genetic differentiation between autosome and sex chromosomes, we oriented White-throated sparrow genome scaffolds by mapping them to the Zebra finch (*Taeniopygia guttata*) chromosomal level assembly using satsuma2 synteny (Grabherr et al. 2010).

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TABLE S1. Alpine and subalpine sites in western Colorado with potential breeding records (1 June-31 July) of Brewer's Sparrows by site type, ownership, county, and site, 1914-2022. Elevation and coordinates are approximate. See map in Figure 1.

Site type	Ownership (Wilderness Area)[†]	County	Site	Elev (m)	Latitude	Longitude
Alpine	Arapaho NF (Mount Evans)	Clear Creek	Chicago Lakes Basin	3417	39.630795	-105.625993
Alpine	Arapaho NF (Mount Evans)	Clear Creek	Mount Goliath	3570	39.628542	-105.599861
Alpine	Arapaho NF/Pike NF	Clear Creek	Guanella Pass	3535	39.595813	-105.717505
Alpine	Arapaho NF	Clear Creek	Mount Evans-Summit Lake	3918	39.600000	-105.641000
Alpine	Arapaho NF/Pike NF	Clear Creek	Mount Evans	4288	39.586707	-105.643158
Alpine	Arapaho NF/Private	Clear Creek	Saint Mary's Glacier	3444	39.834701	-105.651630
Alpine	Arapaho NF	Clear Creek/Grand	Berthoud Pass	3450	39.798022	-105.776862
Alpine	Arapaho NF	Grand	Rollins Pass (W side)	3553	39.922136	-105.686339
Alpine	Bureau of Land Management/Private	Lake/Park	Mosquito Pass	3841	39.271507	-106.191951
Alpine	Gunnison NF	Gunnison	Cumberland Pass	3627	38.705200	-106.477470
Alpine	Gunnison NF	Gunnison	Italian Mountain	3645	38.924831	-106.747084
Alpine	Gunnison NF/Private	Gunnison	Scarp Ridge	3631	38.899476	-107.095660
Alpine	Gunnison NF/White River NF	Gunnison/Pitkin	Taylor Pass	3622	39.018024	-106.758303
Alpine	Pike NF (Mount Evans)	Clear Creek	Bierstadt Trail	3708	39.590656	-105.686533
Alpine	Pike NF	Park	Mudsill Spring	3619	39.214607	-106.123019
Alpine	Pike NF	Park	Pennsylvania Mountain	3844	39.261386	-106.134650
Alpine	Rio Grande NF	Conejos	Long Trek Mountain	3630	37.397305	-106.662489
Alpine	Rio Grande NF	Hinsdale	Jarosa Mesa	3665	37.923138	-107.275157
Alpine	Rio Grande NF	Hinsdale	Kitty Creek	3590	37.848120	-107.309783
Alpine	Rio Grande NF (Weminuche)	Mineral	Red Lakes Trail	3775	37.668949	-107.134162
Alpine	Rio Grande NF	Mineral	Snow Mesa	3666	37.903795	-107.103967
Alpine	Rio Grande NF	Rio Grande	Blowout Pass	3571	37.464000	-106.448000
Alpine	Rio Grande NF	Rio Grande	Grayback Mountain	3671	37.455231	-106.555147
Alpine	Rocky Mountain National Park	Larimer	Flattop Mountain Trail	3479	40.314516	-105.676771
Alpine	Rocky Mountain National Park	Larimer	Lava Cliffs Overlook	3686	40.424914	-105.753416
Alpine	Rocky Mountain National Park	Larimer	Marmot Point	3617	40.442510	-105.737045
Alpine	Rocky Mountain National Park	Larimer	Medicine Bow Curve	3472	40.447974	-105.751632
Alpine	Rocky Mountain National Park	Larimer	Trail Ridge Visitor Center	3591	40.440807	-105.754879
Alpine	Rocky Mountain National Park	Larimer	Ute Trail (western)	3428	40.429391	-105.790223
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Blue Lake	3461	40.087616	-105.617035
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Bald Mountain	3420	39.996782	-105.609590
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Chittenden	3334	39.978651	-105.638683
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Fourth of July Mine	3418	40.010223	-105.656096

[†] NF = National Forest.

TABLE S1 (cont.). Alpine and subalpine sites in western Colorado with potential breeding records (1 June-31 July) of Brewer's Sparrows by site type, ownership, county, and site, 1914-2022. Elevation and coordinates are approximate. See map in Figure 1.

Site type	Ownership (Wilderness Area)[†]	County	Site	Elev (m)	Latitude	Longitude
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Horseshoe Creek	3352	40.011249	-105.596302
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Mt. Audubon Trail – Upper	3561	40.102040	-105.592240
Alpine	Roosevelt NF (Indian Peaks)	Boulder	Mt. Audubon Trail - Lower	3309	40.093289	-105.583620
Alpine	Roosevelt NF	Boulder	Niwot Ridge	3460	40.052170	-105.583738
Alpine	Roosevelt NF (James Peak)	Gilpin	Rollins Pass (E side)	3313	39.926654	-105.661526
Alpine	Roosevelt NF (Rawah)	Larimer	Cameron Peak (SE side)	3465	40.620441	-105.889390
Alpine	Roosevelt NF (Rawah)	Larimer	Cameron Peak (W side)	3577	40.623034	-105.898942
Alpine	Routt NF (Flattops)	Garfield	Bear River Trail	3466	40.002639	-107.165096
Alpine	Routt NF (Flattops)	Garfield	Devil's Causeway	3537	40.039577	-107.148407
Alpine	San Isabel NF (Sangre de Cristo)	Custer	Upper Venable Lake	3753	38.066867	-105.620124
Alpine	San Isabel NF/Pike NF/Private	Lake/Park	Weston Pass	3583	39.132966	-106.181354
Alpine	San Juan NF	La Plata	Endlich Mesa	3725	37.507931	-107.601206
Alpine	San Juan NF	La Plata	Upper Florida River Basin	3637	37.559272	-107.582318
Alpine	San Juan NF	San Juan	Ice Lake Basin	3731	37.810690	-107.806563
Alpine	San Juan NF	San Juan	Jura Knob	3605	37.732282	-107.771360
Alpine	San Juan NF/Rio Grande NF	Rio Grande	Summit Pass	3582	37.427020	-106.659315
Alpine	White River NF (Flattops)	Garfield	Chinese Wall Trail	3492	39.998685	-107.174290
Alpine	White River NF (Flattops)	Garfield	Mosquito Peak	3512	40.004827	-107.172650
Alpine	White River NF (Flattops)	Garfield	West Mountain Trail	3488	39.896364	-107.161762
Alpine	White River NF (Maroon Bells-Snowmass)	Gunnison	Frigid Air Pass	3630	39.049823	-107.019883
Alpine	White River NF (Hunter-Fryingpan)	Pitkin	Independence Lake	3723	39.132157	-106.577358
Alpine	White River NF (Hunter-Fryingpan)	Pitkin/Lake	Independence Pass	3755	39.113971	-106.566830
Alpine	White River NF	Summit	Blue Lakes	3598	39.386690	-106.098770
Alpine	White River NF (Eagle's Nest)	Summit	Dora Mountain	3658	39.784204	-106.303847
Alpine	White River NF (Ptarmigan Peak)	Summit	Ptarmigan Peak	3556	39.675119	-106.038825
Alpine	White River NF/Pike NF	Summit	Hoosier Pass	3634	39.358667	-106.050903
Subalpine	Gunnison NF	Gunnison	Boston Peak	3142	38.835495	-106.750957
Subalpine	Gunnison NF/Private	Gunnison	Robinson Basin	3225	38.887238	-107.116778
Subalpine	Gunnison NF (Maroon Bells-Snowmass)	Gunnison	Rustlers Gulch Trail	3145	39.001612	-107.006166
Subalpine	Pike NF	Park	Lower Weston Pass Road	3145	39.085970	-106.142274
Subalpine	Rocky Mountain National Park	Larimer	Old Fall River Road	3247	40.428917	-105.726929
Subalpine	Roosevelt NF	Boulder	Brainerd Lake	3163	40.077887	-105.574540
Subalpine	Roosevelt NF	Boulder	Caribou Town Site	3047	39.981050	-105.579289
Subalpine	Roosevelt NF (Indian Peaks)	Boulder	Lake Isabelle Trail	3208	40.072226	-105.592046
Subalpine	Roosevelt NF	Gilpin	Elk Park	3133	39.865820	-105.591651

[†] NF = National Forest.

TABLE S1 (cont.). Alpine and subalpine sites in western Colorado with potential breeding records (1 June-31 July) of Brewer's Sparrows by site type, ownership, county, and site, 1914-2022. Elevation and coordinates are approximate. See map in Figure 1.

Site type	Ownership (Wilderness Area)[†]	County	Site	Elev (m)	Latitude	Longitude
Subalpine	Routt NF	Garfield	Stillwater Reservoir	3170	40.025445	-107.127428
Subalpine	Routt NF	Jackson	Buffalo Pass-Summit Lake	3146	40.544803	-106.681978
Subalpine	Routt NF (Mount Zirkel)	Jackson	Rainbow Lake	3109	40.648515	-106.628721
Subalpine	Routt NF (Mount Zirkel)	Routt	Gold Creek	2940	40.786979	-106.684355
Subalpine	San Juan NF	Dolores	Cross Mountain Trailhead	3063	37.797627	-107.937498
Subalpine	San Juan NF	San Juan	Molas Lake	3207	37.748729	-107.683868
Subalpine	San Juan NF	San Juan	Molas Pass	3323	37.737450	-107.697333
Subalpine	Uncompaghre NF	Ouray	Crystal Lake	2909	37.959545	-107.662399
Subalpine	Uncompahgre NF	San Miguel	Lizard Head Pass	3121	37.811263	-107.905682
Subalpine	White River NF	Garfield	Coffee Pot Springs	3095	39.681332	-107.198171
Subalpine	White River NF	Garfield	Crane Park	3152	39.703255	-107.246690
Subalpine	White River NF	Garfield	Crescent Lake	3293	39.908379	-107.155181
Subalpine	White River NF	Garfield	White Owl Lake	3261	39.746100	-107.300666
Subalpine	White River NF (Maroon Bells-Snowmass)	Pitkin	Thomas Lakes	3113	39.273651	-107.141716

[†] NF = National Forest.

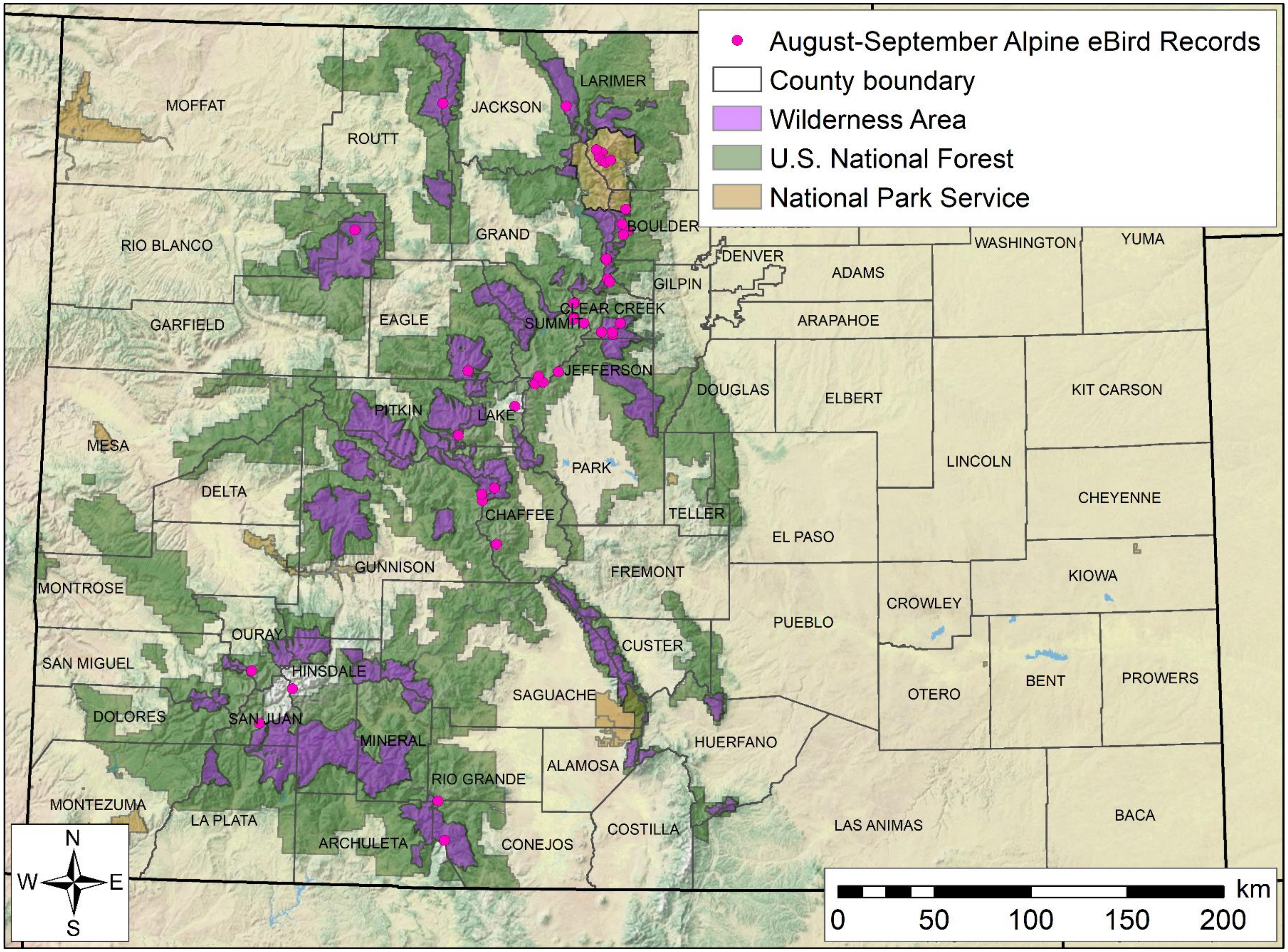

FIGURE S1. Alpine sites where eBird contributors have reported Brewer's Sparrows (*Spizella breweri*) in western Colorado in August-September (1999-2022). See Table S2 for site details.

TABLE S2. Summary of alpine sites where eBird contributors have reported Brewer's Sparrows in western Colorado in August-September (1999-2022), by ownership, county, and site. Location coordinates and elevation are approximate. See map in Figure S1.

Site	Ownership[†] (Wilderness Area)	County	Elev (m)	Latitude	Longitude
Grays and Torreys Peaks	Arapaho NF	Clear Creek	4159	39.639042	-105.818810
Guanella Pass[‡]	Arapaho NF	Clear Creek	3542	39.596772	-105.710239
James Peak	Arapaho NF (James Peak)	Clear Creek	3700	39.846022	-105.677278
Kingston Peak Trail	Arapaho NF	Clear Creek	3542	39.831596	-105.663857
Loveland Pass	Arapaho NF/White River NF	Clear Creek/Summit	3648	39.664585	-105.878922
Mount Goliath[‡]	Arapaho NF	Clear Creek	3641	39.640000	-105.597000
Woods Creek Trail	Arapaho NF	Clear Creek	3724	39.732941	-105.875521
Rollins Pass (W side)[‡]	Arapaho NF	Grand	3564	39.933894	-105.684614
American Basin	Bureau of Land Management	Hinsdale	3759	37.908640	-107.518329
Cottonwood Pass	Gunnison NF	Gunnison	3526	38.834718	-106.418901
Boreas Pass	Pike NF/White River NF	Park/Summit	3499	39.410004	-105.968027
Mount Evans-Summit Lake[‡]	Arapaho NF	Clear Creek	3912	39.597730	-105.644500
Mount Evans[‡]	Pike NF	Clear Creek	4288	39.586707	-105.643158
Mount Lincoln	Pike NF	Park	4273	39.352313	-106.108872
Lake County Road 38	Private	Lake	3468	39.246371	-106.228591
Crater Lake Trail Junction	Rio Grande NF	Rio Grande	3548	37.399594	-106.648775
Mount Chapin	Rocky Mountain National Park	Larimer	3544	40.435264	-105.717442
Medicine Bow Curve[‡]	Rocky Mountain National Park	Larimer	3472	40.447974	-105.751632
Ute Trail (eastern)	Rocky Mountain National Park	Larimer	3531	40.389825	-105.693590
Rainbow Curve	Rocky Mountain National Park	Larimer	3279	40.399800	-105.663795
Forest Canyon Overlook	Rocky Mountain National Park	Larimer	3563	40.395620	-105.712626
Tundra Communities Trail	Rocky Mountain National Park	Larimer	3700	40.412569	-105.733018
Little Molas Lake	San Juan NF	San Juan	3327	37.743707	-107.708834
Hoosier Pass[‡]	Pike NF/White River NF	Park/Summit	3540	39.360357	-106.059489
East Fork Navajo River Basin	Rio Grande NF (South San Juan)	Conejos	3584	37.216755	-106.605801
King Lake	Roosevelt NF (Indian Peaks)	Boulder	3512	39.939693	-105.685670
Left Hand Reservoir	Roosevelt NF	Boulder	3230	40.070026	-105.555844
Mount Audubon Trail[‡]	Roosevelt NF (Indian Peaks)	Boulder	3561	40.102040	-105.592240
Niwot Ridge[‡]	Roosevelt NF	Boulder	3457	40.052170	-105.583738
St. Vrain Mountain	Roosevelt NF (Indian Peaks)	Boulder	3386	40.170937	-105.568921

[†] NF = National Forest.

[‡] Sites that also had records in June-July.

TABLE S2 (cont.). Summary of alpine sites where eBird contributors have reported Brewer's Sparrows in western Colorado in August-September (1999-2022), by ownership, county, and site. Location coordinates and elevation are approximate. See map in Figure S1.

Site	Ownership[†] (Wilderness Area)	County	Elev (m)	Latitude	Longitude
Twin Crater Lakes	Roosevelt NF (Rawah)	Jackson	3358	40.649589	-105.939960
Upper Crags Trail	Routt NF (Mount Zirkel)	Routt	3478	40.654146	-106.695315
Lost Lake	San Isabel NF	Chaffee	3612	38.805190	-106.415243
Section 8 Lake	San Isabel NF	Chaffee	3682	38.601588	-106.326858
Brown's Pass Trail	San Isabel NF (Collegiate Peaks)	Chaffee	3711	38.862897	-106.343246
Yankee Boy Basin	Uncompahgre NF	Ouray	3430	37.986760	-107.763176
Blue Lakes[‡]	White River NF	Summit	3482	39.388150	-106.088461
Fancy Pass	White River NF (Holy Cross)	Eagle	3621	39.409241	-106.513867
Lost Lakes Peaks	White River NF	Garfield	3529	40.054883	-107.215322
Loveland Pass-Pass Lake	White River NF	Summit	3605	39.655397	-105.878455
Independence Pass[‡]	White River NF/ San Isabel NF	Pitkin/Lake	3689	39.108326	-106.564318

[†] NF = National Forest.

[‡] Sites that also had records in June-July.

TABLE S3. Sites surveyed for Brewer’s Sparrows by Colorado Parks and Wildlife (CPW), volunteers, or birders in western Colorado, May-July 2021-2023 by site type, ownership, county, and site. * = Brewer’s Sparrows detected. See map in Figure 2.

Site type	Ownership[†]	County	Site[†]	Elev (m)	Latitude	Longitude
Alpine	Arapaho NF	Clear Creek	Jones Pass	3574	39.782458	-105.880568
Alpine	Arapaho NF	Clear Creek	Kelso Mountain*	3647	39.593553	-105.720462
Alpine	Arapaho/Pike NF	Clear Creek	Guanella Pass*	3523	39.595813	-105.717505
Alpine	Arapaho/White River NF	Clear Creek/Summit	Loveland Pass	3581	39.661388	-105.876710
Alpine	Arapaho NF	Grand	Rollins Pass*	3553	39.922136	-105.686339
Alpine	BLM	Hinsdale	Rambouillet Park*	3537	37.946375	-107.256975
Alpine	BLM	Lake/Park	Mosquito Pass	3593	39.271507	-106.191951
Alpine	Gunnison NF	Gunnison	Cumberland Pass*	3676	38.705200	-106.477470
Alpine	Gunnison NF	Gunnison	Italian Mountain*	3665	38.924831	-106.747084
Alpine	Gunnison NF	Gunnison	Scarp Ridge*	3677	38.899476	-107.095660
Alpine	Gunnison/White River NF	Gunnison/Pitkin	Taylor Pass*	3615	39.018024	-106.758303
Alpine	Pike NF	Park	Cone Peaks	3655	39.425927	-105.694783
Alpine	Rio Grande NF	Conejos	Tobacco Lake	3668	37.295069	-106.559896
Alpine	Rio Grande NF	Hinsdale	Buck Mountain*	3674	37.926251	-107.223251
Alpine	Rio Grande NF	Hinsdale	Jarosa Mesa*	3530	37.923138	-107.275157
Alpine	Rio Grande NF	Hinsdale	Kitty Creek	3557	37.848120	-107.309783
Alpine	Rio Grande NF	Mineral	Bristol Head*	3568	37.812199	-107.072543
Alpine	Rio Grande NF	Mineral	Snow Mesa	3764	37.903795	-107.103967
Alpine	Rio Grande NF	Rio Grande	Blowout Pass	3576	37.464000	-106.448000
Alpine	Rio Grande NF	Rio Grande	Grayback Mountain	3682	37.455231	-106.555147
Alpine	Rio Grande NF	Rio Grande	North Mountain*	3754	37.452894	-106.590144
Alpine	Rio Grande NF	Saguache/Rio Grande	La Garita Cutoff*	3636	37.831273	-106.658356
Alpine	Rio Grande NF	Saguache/Rio Grande	La Garita Stock Driveway*	3734	37.864223	-106.667862
Alpine	Rio Grande NF	San Juan	Stony Pass*	3737	37.790186	-107.542045
Alpine	Rocky Mountain NP	Larimer	Flattop Mountain Trail	3479	40.314516	-105.676771
Alpine	Rocky Mountain NP	Larimer	Ute Trail (western)*	3541	40.439075	-105.762363
Alpine	Roosevelt NF	Boulder	Niwot Ridge*	3395	40.052249	-105.559270
Alpine	Roosevelt NF	Boulder	Niwot Ridge*	3460	40.056158	-105.564849
Alpine	Roosevelt NF	Gilpin	Heart Lake*	3449	39.875944	-105.693139
Alpine	Roosevelt NF	Larimer	Cameron Peak/Blue Lake	3537	40.623034	-105.898942
Alpine	Routt NF	Garfield	Devil's Causeway*	3484	40.039577	-107.148407
Alpine	Routt NF	Jackson/Routt	Lost Ranger Peak*	3507	40.675553	-106.684127
Alpine	Routt NF	Park	Swamp Park	3338	40.816593	-106.650267
Alpine	San Isabel NF	Huerfano/Custer	Greenhorn Mountain	3477	37.947428	-105.086804
Alpine	San Isabel NF	Lake	Mountain Boy Basin*	3668	39.091905	-106.575690

[†] SWA = State Wildlife Area, NF = National Forest, NP = National Park, BLM = Bureau of Land Management.

TABLE S3 (cont.). Sites surveyed for Brewer's Sparrows by Colorado Parks and Wildlife (CPW), volunteers, or birders in western Colorado, May-July 2021-2023 by site type, ownership, county, and site. * = Brewer's Sparrows detected. See map in Figure 2.

Site type	Ownership[†]	County	Site[†]	Elev (m)	Latitude	Longitude
Alpine	San Isabel/Pike NF	Lake/Park	Weston Pass*	3582	39.141951	-106.193249
Alpine	San Juan NF	La Plata	Kennebec Pass*	3498	37.445205	-108.010609
Alpine	White River NF	Summit	Eisenhower Tunnel*	3512	39.688246	-105.930863
Alpine	White River/Pike NF	Summit	Hoosier Pass*	3674	39.358667	-106.050903
Alpine	White River/San Isabel NF	Pitkin/Lake	Independence Pass*	3754	39.113971	-106.566830
Subalpine	Rio Grande NF	Hinsdale	Spring Creek Pass*	3299	37.925650	-107.159930
Subalpine	San Juan NF	San Juan	Molas Pass*	3303	37.737450	-107.697333
Subalpine	White River NF	Garfield	Crane Park*	3159	39.703255	-107.246690
Subalpine	White River NF	Garfield	White Owl Lake*	3245	39.746100	-107.300666
Subalpine	White River NF	Pitkin	Thomas Lakes Trail*	2837	39.273651	-107.141716
Sagebrush	BLM	Alamosa	Hwy 150*	2373	37.631887	-105.592883
Sagebrush	BLM	Alamosa	San Luis Valley-South*	2321	37.516513	-105.623711
Sagebrush	BLM	Delta	Crawford Road*	1746	38.806097	-107.630230
Sagebrush	BLM	Garfield	Rifle Arch Trailhead*	1794	39.613292	-107.808033
Sagebrush	BLM	Grand	Kremmling*	2460	40.089689	-106.188812
Sagebrush	BLM	Gunnison	Parlin*	2552	38.504251	-106.659829
Sagebrush	BLM	Gunnison	Sapinero Mesa*	2545	38.397599	-107.203616
Sagebrush	BLM	Jackson	Lake John SWA*	2477	40.793191	-106.472618
Sagebrush	BLM	Lake	Twin Lakes*	2825	39.132779	-106.331758
Sagebrush	BLM	Mesa	Piñon Mesa*	2101	38.946128	-108.945030
Sagebrush	BLM	Moffat	Axial Basin*	1941	40.363754	-107.819895
Sagebrush	BLM	Saguache	Cochetopa*	2735	38.276100	-106.738000
Sagebrush	BLM	Saguache	Del Norte	2444	37.788352	-106.301784
Sagebrush	BLM	Saguache	Poncha Pass*	2645	38.375925	-106.053445
Sagebrush	BLM	Saguache	San Luis Valley-North*	2381	38.186139	-105.877322
Sagebrush	CPW	Alamosa	San Luis Lakes SWA*	2296	37.676195	-105.735751
Sagebrush	CPW	San Miguel	Dry Creek SWA*	2065	38.051378	-108.499988
Sagebrush	CPW	San Miguel	Dan Noble SWA	2348	37.971606	-108.340001
Sagebrush	Grand Mesa NF	Mesa	Indian Point*	3042	38.910481	-108.176269
Sagebrush	Grand Mesa NF	Mesa	Land's End*	3038	39.028955	-108.220631
Sagebrush	Pike NF	Park	Long Park	2937	39.058235	-106.018268
Sagebrush	Pike NF	Park	Tarryall Reservoir SWA	2702	39.229012	-105.610832
Sagebrush	Routt NF	Routt	California Park*	2468	40.738065	-107.118901
Sagebrush	White River NF	Eagle	Camp Hale South*	2826	39.426182	-106.319161
Sagebrush	White River NF	Eagle	Muddy Creek Road*	2442	39.763342	-106.623821
Sagebrush	White River NF	Summit	Green Mountain Reservoir*	2449	39.877929	-106.281118

[†] SWA = State Wildlife Area, NF = National Forest, NP = National Park, BLM = Bureau of Land Management.

TABLE S4. Summary of results from surveys at sagebrush and alpine sites for Brewer's Sparrows by Colorado Parks and Wildlife in Colorado by survey date, May-July 2021-2023.

Site type	Site[†]	Survey date	No. males detected	No. males recorded	No. captured M, F, U	Breeding status
Sagebrush	Piñon Mesa	05/05/21	3	3	2, 0, 0	Probable
Sagebrush	Dan Noble SWA	05/07/21	0	0		
Sagebrush	Dry Creek SWA	05/07/21	4	2	4, 0, 0	Probable
Sagebrush	Del Norte	05/11/21	0	0		
Sagebrush	San Luis Lakes SWA	05/12/21	10	8	0, 0, 0	Probable
Sagebrush	San Luis Valley-South	05/12/21	1	0	0, 0, 0	Possible
Sagebrush	San Luis Valley-North	05/12/21	1	1	0, 0, 0	Possible
Sagebrush	Hwy. 150	05/12/21	2	2	2, 0, 0	Probable
Sagebrush	Poncha Pass	05/12/21	1	0	1, 0, 0	Possible
Sagebrush	Poncha Pass[‡]	05/13/21	12	10	3, 0, 0	Probable
Sagebrush	Tarryall Reservoir SWA	05/13/21	0	0		
Sagebrush	Long Park	05/13/21	0	0		
Sagebrush	Twin Lakes	05/14/21	3	2	1, 0, 1	Probable
Sagebrush	Green Mountain Reservoir	05/15/21	13	12	3, 0, 0	Probable
Sagebrush	Kremmling	05/16/21	12	11	6, 0, 0	Probable
Sagebrush	Muddy Creek Road	05/17/21	4	3	2, 0, 0	Probable
Sagebrush	Rifle Arch Trailhead	05/19/21	5	3	0, 0, 0	Probable
Sagebrush	Axial Basin	05/19/21	9	5	2, 0, 0	Probable
Sagebrush	Parlin	05/19/21	10	9	0, 0, 0	Probable
Sagebrush	Sapinero Mesa	05/19/21	8	3	0, 0, 0	Probable
Sagebrush	Lake John SWA	05/20/21	24	23	5, 1, 0	Probable
Sagebrush	California Park	05/21/21	17	14	2, 0, 0	Probable
Sagebrush	Crawford Road	05/22/21	7	7	0, 0, 0	Probable
Sagebrush	Cochetopa	05/23/21	3	1	0, 0, 0	Possible
Sagebrush	Parlin[‡]	05/24/21	15	10	3, 0, 0	Probable
Sagebrush	Sapinero Mesa[‡]	05/24/21	1	1	3, 0, 0	Probable
Sagebrush	Land's End	06/05/21	7	6	2 , 0, 0	Probable
Sagebrush	Camp Hale South	06/18/21	1	0	0, 0, 0	Possible
Sagebrush	Indian Point	06/27/22	8	0	0, 0, 0	Probable
Sagebrush	TOTAL		181	136	41, 1, 1	
Alpine	Cumberland Pass	06/04/21	0	0		
Alpine	Scarp Ridge	06/05/21	0	0		
Alpine	Buck Mountain	06/09/21	2	1	0, 0, 0	Possible
Alpine	Kitty Creek	06/10/21	0	0		
Alpine	Snow Mesa	06/10/21	0	0		
Alpine	Grayback Mountain	06/11/21	0	0		
Alpine	Cumberland Pass[‡]	06/15/21	2	1	1, 0, 0	Probable
Alpine	Italian Mountain	06/15/21	2	1	1, 0, 0	Probable

[†] SWA = State Wildlife Area.

[‡] Site also visited on an earlier date. Males likely detected on an earlier visit were excluded from the total for all sites combined.

TABLE S4 (cont.). Summary of results from surveys at sagebrush and alpine sites for Brewer's Sparrows by Colorado Parks and Wildlife in Colorado by survey date, May-July 2021-2023.

Site type	Site	Survey date	No. males detected	No. males recorded	No. captured M, F, U	Breeding status
Alpine	Scarp Ridge[‡]	06/16/21	1	1	1, 0, 0	Probable
Alpine	Mosquito Pass	06/17/21	0	0		
Alpine	Weston Pass	06/17/21	2	2	0, 0, 0	Possible
Alpine	Weston Pass[‡]	06/18/21	2	2	2, 0, 0	Probable
Alpine	Independence Pass	06/18/21	4	4	3, 0, 0	Probable
Alpine	Italian Mountain[‡]	06/21/21	5	2	1, 0, 0	Probable
Alpine	Taylor Pass	06/21/21	8	5	4, 0, 0	Probable
Alpine	Guanella Pass	06/22/21	11	6	5, 0, 0	Probable
Alpine	Rollins Pass	06/23/21	11	7	6, 1, 0	Probable
Alpine	Guanella Pass[‡]	06/24/21	6	2	1, 0, 0	Probable
Alpine	Hoosier Pass	06/24/21	3	3	2, 0, 0	Probable
Alpine	Devil's Causeway	06/28/21	11	8	3, 0, 0	Probable
Alpine	Swamp Park	06/29/21	0	0		
Alpine	Blue Lake	06/30/21	0	0		
Alpine	Grayback Mountain[‡]	07/01/21	0	0		
Alpine	Blowout Pass	07/01/21	0	0		
Alpine	Buck Mountain[‡]	07/02/21	3	0	3, 0, 0	Probable
Alpine	Jarosa Mesa	07/02/21	10	5	8, 0, 0	Probable
Alpine	Niwot Ridge	07/10/21	2	0	0, 0, 0	Possible
Alpine	Bristol Head	06/30/22	3	0	0, 0, 0	Possible
Alpine	Eisenhower Tunnel	07/22/23	0	0		
Alpine	Guanella Pass[‡]	07/23/23	1	0	0, 0, 0	Confirmed
Alpine	Hoosier Pass[‡]	07/26/23	1	0	0, 0, 0	Confirmed
Alpine	TOTAL		78	50	41, 1, 0	

[†] SWA = State Wildlife Area.

[‡] Site also visited on an earlier date. Males likely detected on an earlier visit were excluded from the total for all sites combined.

TABLE S5. Summary of results for alpine and subalpine sites surveyed for Brewer's Sparrows by Colorado Parks and Wildlife volunteers, National Park Service volunteers, or cooperating birders in June-July 2021-2023 in western Colorado by site type, survey date (month-day), and site.

Site type	Survey date	Site[†]	No. detected M, F, U, J	Breeding status
Alpine	06/10/21	Kennebec Pass	0, 0, 0, 0	
Alpine	06/23/21	Flattop Mountain Trail	0, 0, 0, 0	
Alpine	06/23/21	Ute Trail (western)	1, 0, 0, 0	Possible
Alpine	07/04/22	Eisenhower Tunnel	1, 0, 0, 0	Possible
Alpine	07/04/22	Loveland Pass	0, 0, 0, 0	
Alpine	07/07/22	Jones Pass	0, 0, 0, 0	
Alpine	07/08/22	Cone Peaks	0, 0, 0, 0	
Alpine	07/08/22	Niwot Ridge[†]	1, 0, 0, 0	Possible
Alpine	07/10/21	Independence Pass[†]	6, 0, 0, 0	Probable
Alpine	07/10/21	Kelso Mountain	1, 0, 0, 0	Possible
Alpine	07/11/21	Kennebec Pass[†]	2, 0, 0, 0	Possible
Alpine	07/12/21	Kennebec Pass[†]	1, 0, 0, 0	Possible
Alpine	07/14/22	La Garita Stock Driveway	7, 0, 0, 0	Probable
Alpine	07/15/22	Greenhorn Mountain	0, 0, 0, 0	
Alpine	07/17/22	La Garita Cutoff	1, 0, 0, 0	Possible
Alpine	07/17/21	Rollins Pass[†]	8, 0, 0, 0	Confirmed
Alpine	07/18/22	Tobacco Lake	0, 0, 0, 0	
Alpine	07/19/21	Heart Lake	1, 0, 0, 0	Possible
Alpine	07/23/22	Lost Ranger Peak	1, 0, 0, 0	Possible
Alpine	07/24/23	Rambouillet Park	0, 0, 2, 0	Possible
Alpine	07/02/23	Mountain Boy Basin	1, 0, 0, 0	Possible
Alpine	07/04/23	Mountain Boy Basin[†]	1, 0, 0, 0	Possible
Alpine	07/11/23	Mountain Boy Basin[†]	1, 0, 0, 0	Possible
Alpine	07/24/23	North Mountain	0, 0, 0, 3	Possible
Alpine	07/26/23	Stony Pass	0, 0, 1, 1	Possible
Alpine		TOTAL	19, 0, 3, 3	
Subalpine	06/29/21	Thomas Lakes Trail	3, 0, 0, 0	Possible
Subalpine	07/01/21	Spring Creek Pass	1, 0, 0, 0	Possible
Subalpine	07/10/21	Crane Park	10, 0, 0, 0	Probable
Subalpine	07/10/21	White Owl Lake	1, 0, 0, 0	Possible
Subalpine	07/13/21	Molas Pass	1, 0, 0, 0	Possible
Subalpine		TOTAL	16, 0, 0	

[†] Site also visited on an earlier date. Males likely detected on an earlier visit were excluded from the total for all sites combined.

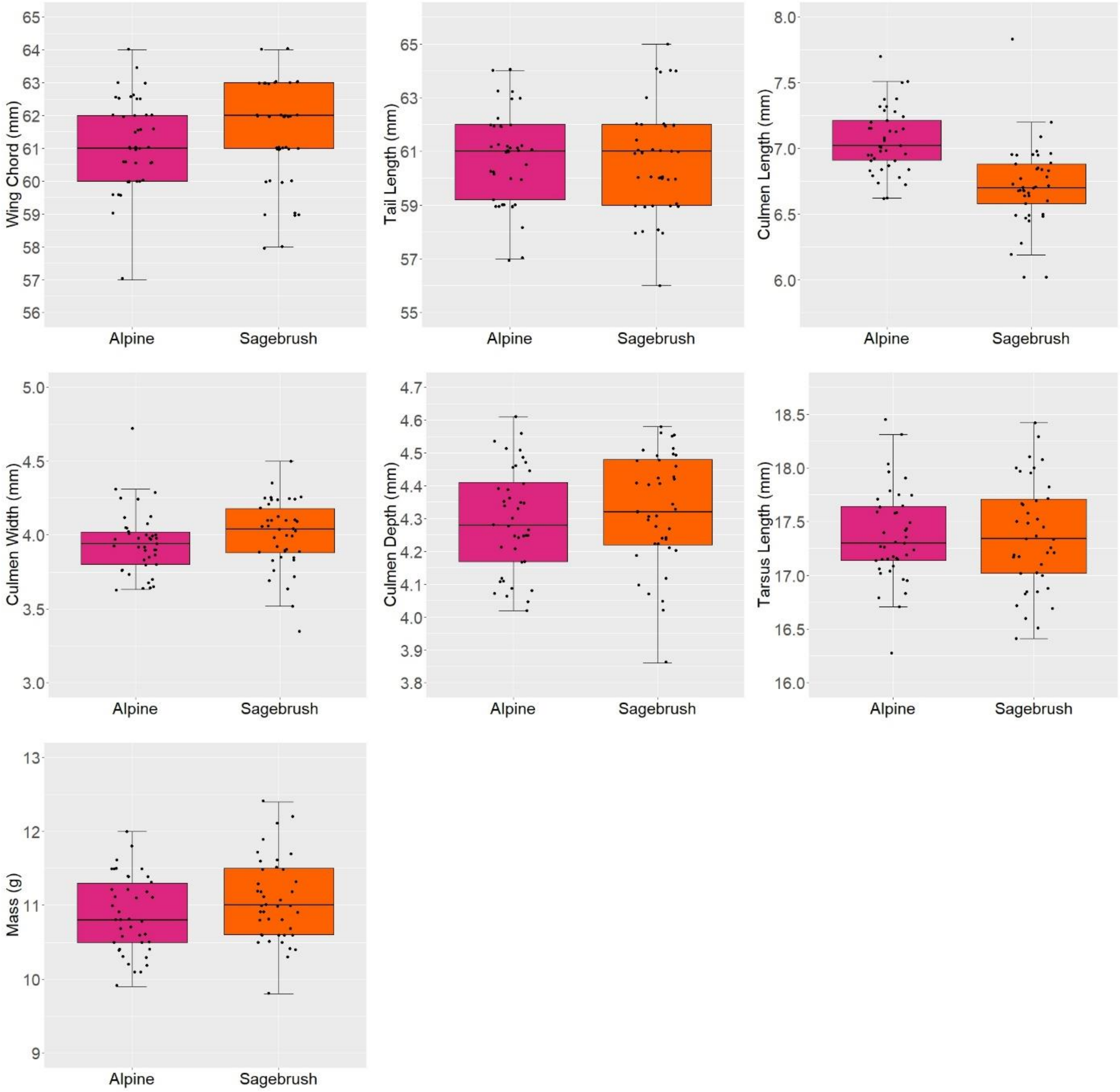

FIGURE S2. Jittered boxplots showing the median and interquartile range for seven external morphological measurements (excluding outliers, see Methods) of male Brewer's Sparrows at alpine (pink; n = 41) and sagebrush (orange; n = 41) sites in western Colorado, 2021.

TABLE S6. Eigenvalues and percent variance explained by each dimension from principal component analysis of 7 external morphological variables measured on male Brewer's Sparrows captured at alpine (n = 41) and sagebrush (n = 41) sites in western Colorado, 2021.

Dimension	Eigenvalue	% of variance	Cumulative %
1	1.93	27.5	27.5
2	1.47	20.9	48.5
3	1.07	15.2	63.7
4	0.90	12.9	76.6
5	0.79	11.3	87.9
6	0.56	8.1	96.0
7	0.28	4.0	100.0

TABLE S7. Rotated loadings from principal component analysis of 7 external morphological variables measured on male Brewer's Sparrows captured at sagebrush (n = 41) and alpine (n = 41) sites in western Colorado, 2021.

Variable	PC1	PC2
Wing chord (mm)	-0.499	0.495
Tail length (mm)	-0.472	0.506
Culmen length (mm)	-0.087	-0.284
Culmen width (mm)	-0.382	-0.412
Culmen depth (mm)	-0.449	-0.355
Tarsus length (mm)	-0.288	-0.282
Mass (g)	-0.301	-0.210

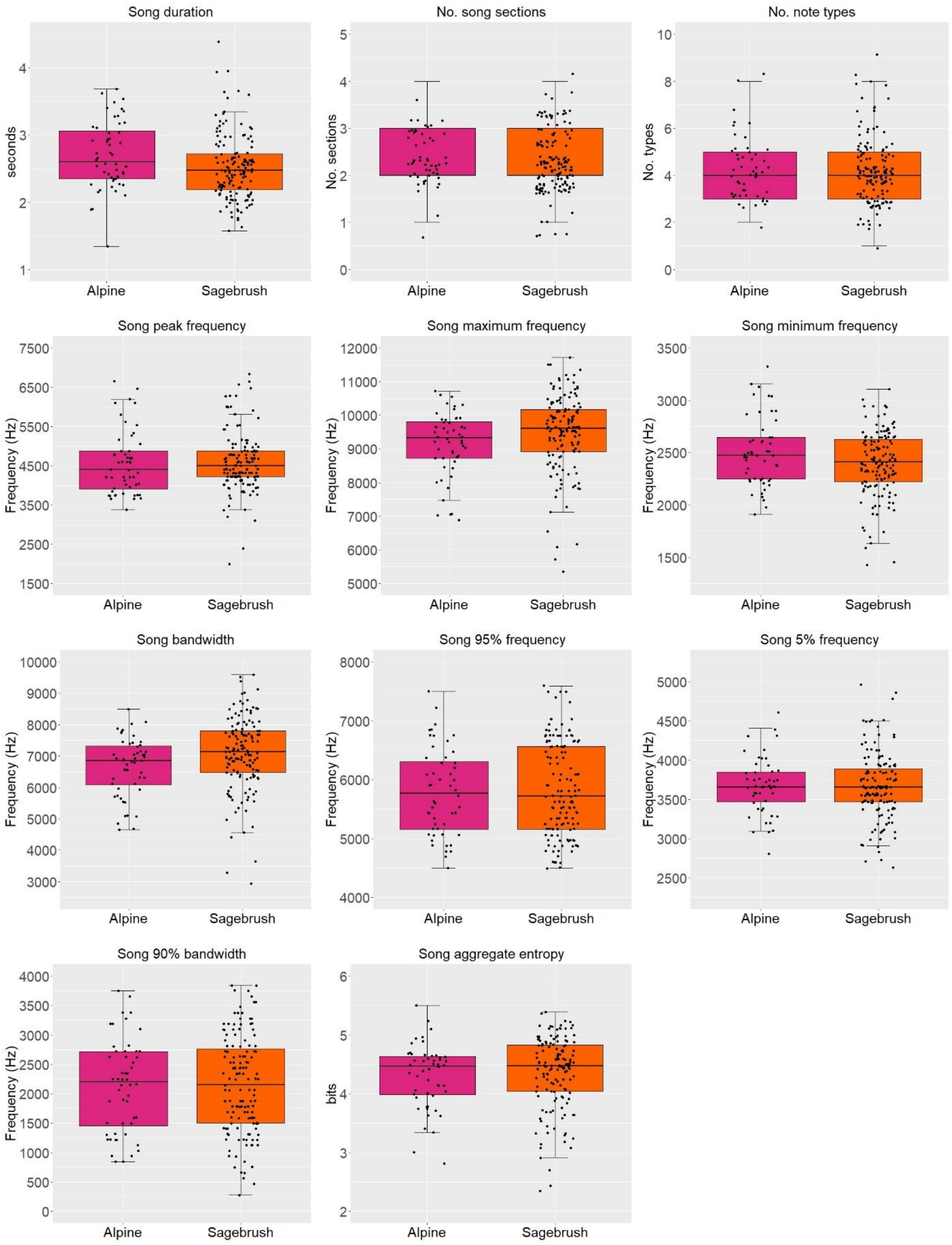

FIGURE S3. Jittered boxplots showing medians and interquartile ranges of song-level acoustic variables measured on Brewer’s Sparrow short songs at alpine (pink; n = 52 short songs from 49 males) and sagebrush (orange; n = 143 songs from 134 males) sites in western Colorado, 2021.

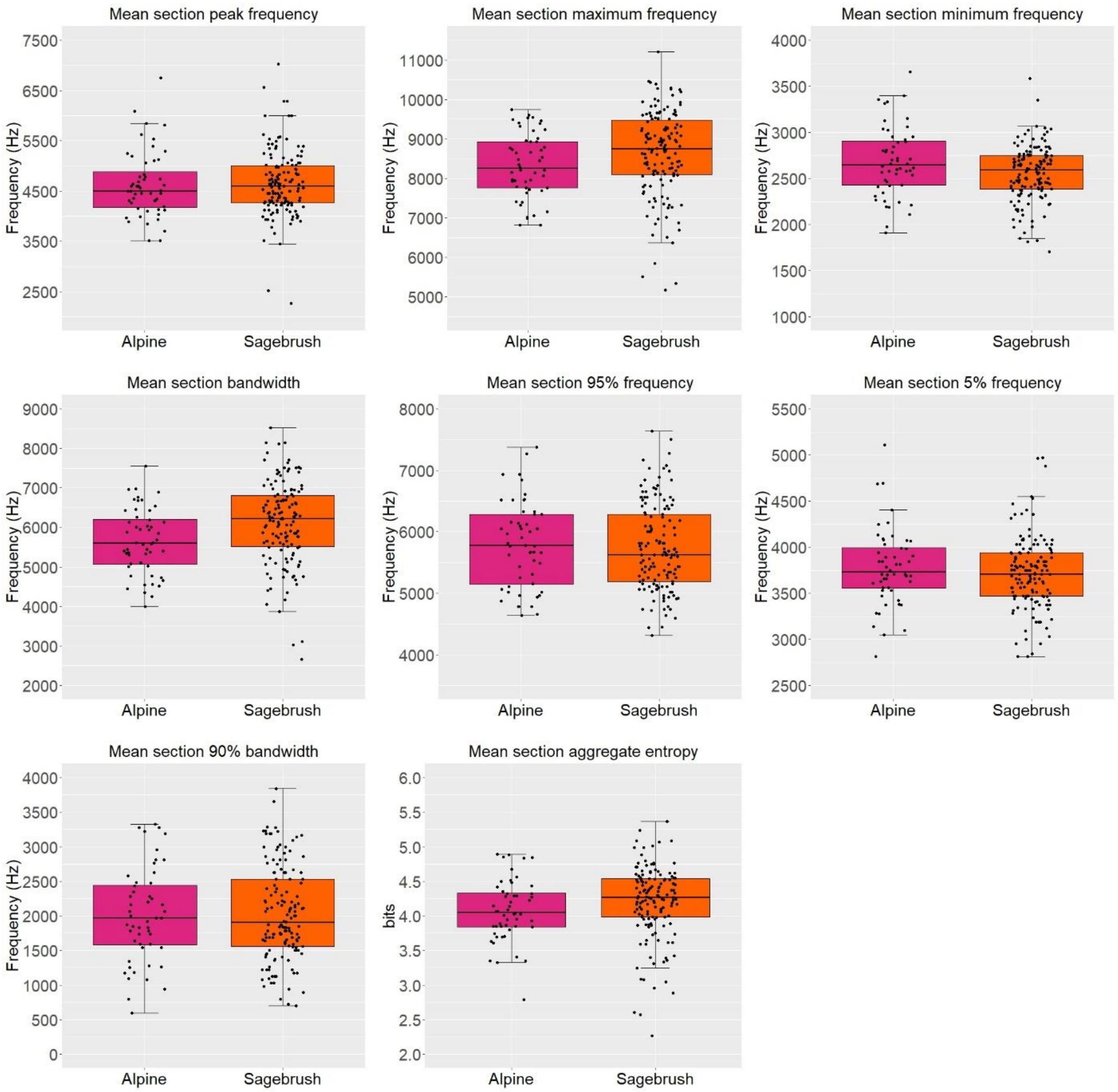

FIGURE S4. Jittered boxplots showing medians and interquartile ranges of section-level acoustic variables measured on Brewer's Sparrow short songs at alpine (pink; n = 52 short songs from 49 males) and sagebrush (orange; n = 143 songs from 134 males) sites in western Colorado, 2021.

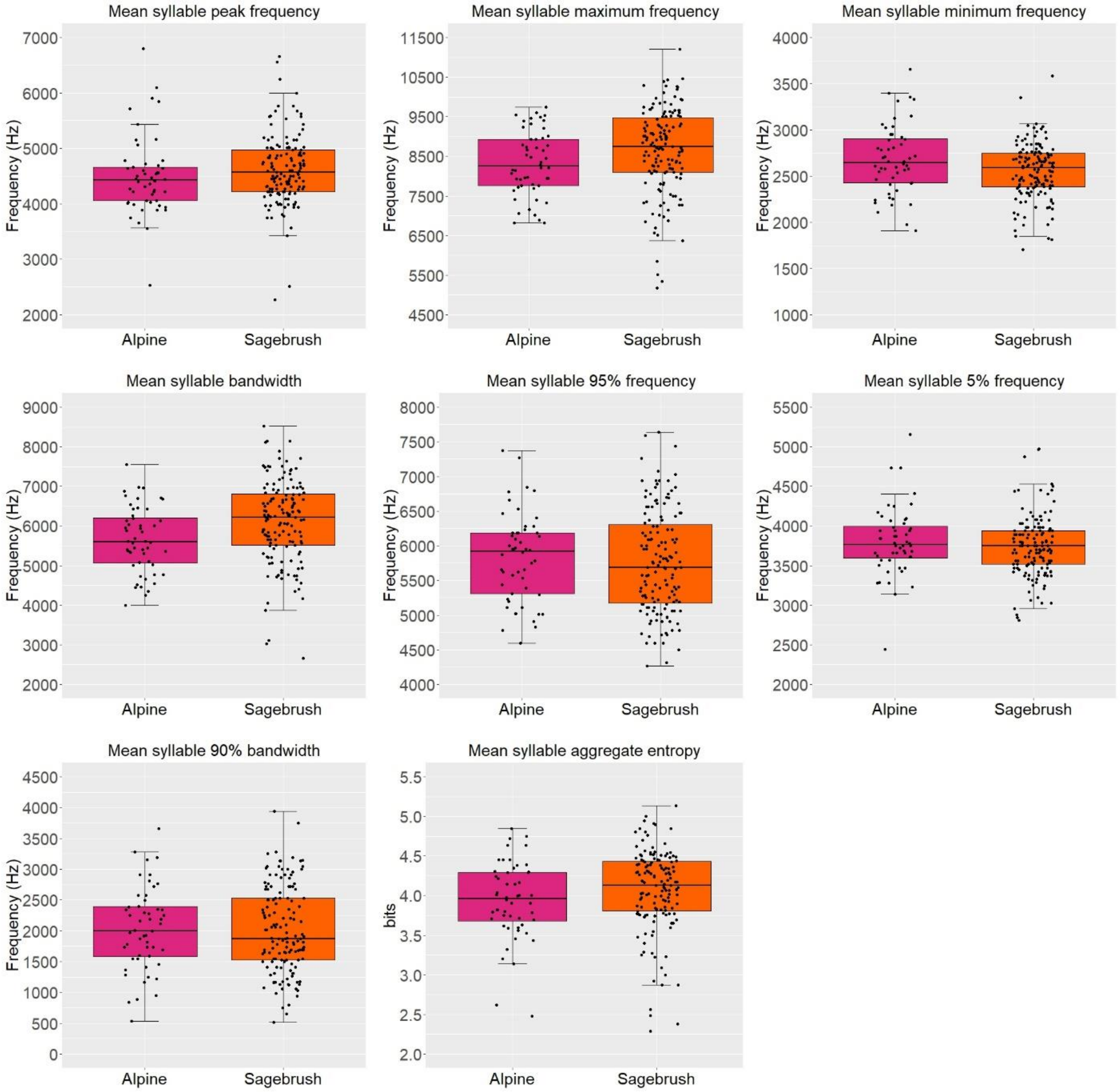

FIGURE S5. Jittered boxplots showing medians and interquartile ranges of syllable-level acoustic variables measured on Brewer’s Sparrow short songs at alpine (pink; n = 52 short songs from 49 males) and sagebrush (orange; n = 143 songs from 134 males) sites in western Colorado, 2021.

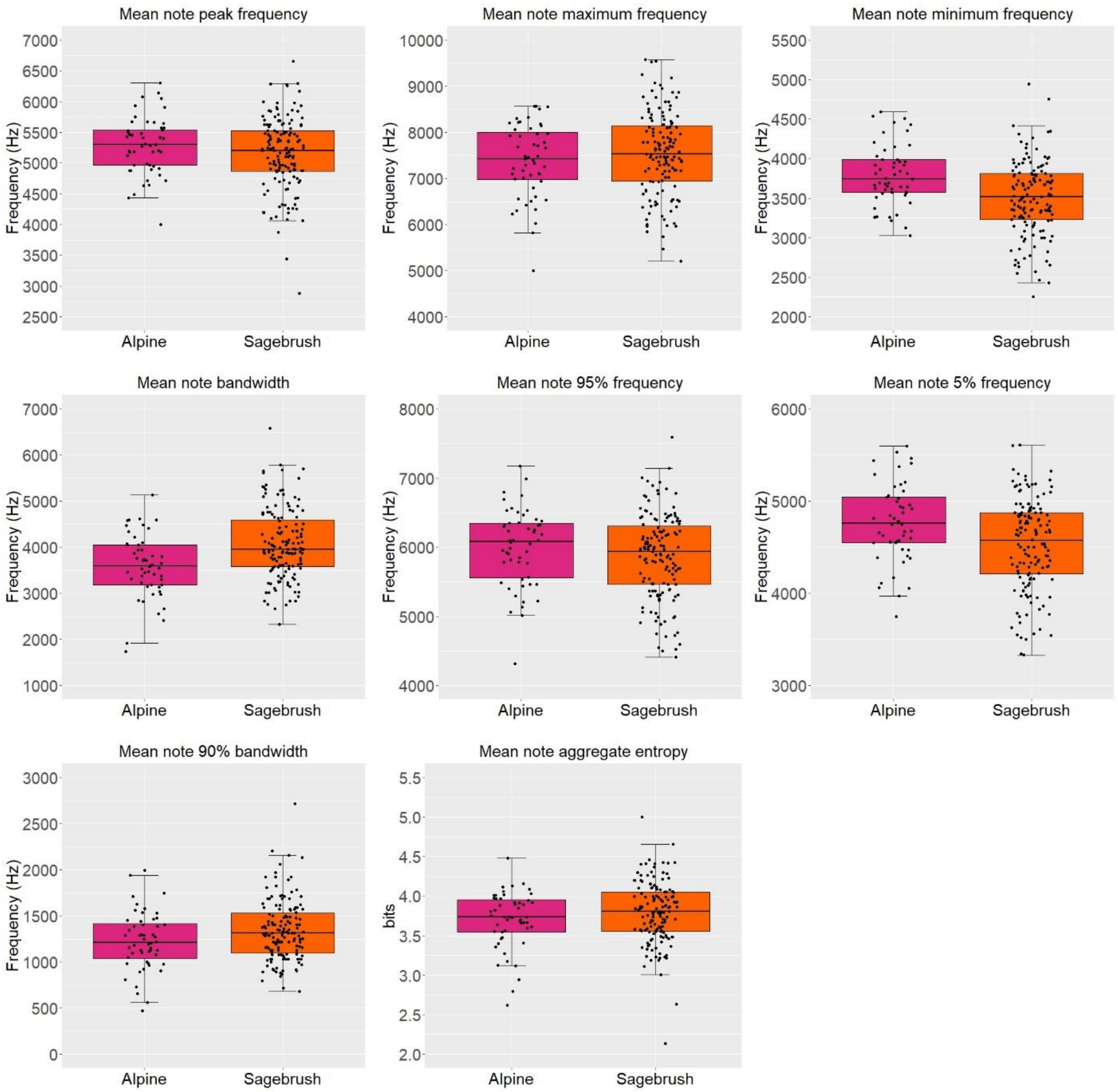

FIGURE S6. Jittered boxplots showing medians and interquartile ranges of note-level acoustic variables measured on Brewer's Sparrow short songs at alpine (pink; n = 52 short songs from 49 males) and sagebrush (orange; n = 143 songs from 134 males) sites in western Colorado, 2021.

TABLE S8. Eigenvalues and percent variance explained by each dimension from principal component analysis of 10 acoustic variables measured on short songs of male Brewer's Sparrows at alpine (n = 52 short songs from 49 males) and sagebrush (n = 143 songs from 134 males) sites in western Colorado, 2021.

Dimension	Eigenvalue	% of variance	Cumulative %
1	5.54	55.4	55.4
2	1.52	15.2	70.6
3	1.19	11.9	82.4
4	0.85	8.6	91.0
5	0.35	3.5	94.5
6	0.25	2.5	97.0
7	0.24	2.4	99.5
8	0.05	0.6	100.0
9	0.00	0.0	100.0
10	0.00	0.0	100.0

TABLE S9. Rotated loadings from principal component analysis of 10 acoustic variables measured on short songs of male Brewer's Sparrows at alpine (n = 52 short songs from 49 males) and sagebrush (n = 143 songs from 134 males) sites in western Colorado, 2021.

Variable	PC1	PC2
Song duration (sec)	-0.014	0.033
Song bandwidth (Hz)	-0.349	-0.145
Mean section maximum frequency (Hz)	-0.412	0.052
Mean section bandwidth (Hz)	-0.407	-0.059
Mean syllable maximum frequency (Hz)	-0.412	0.052
Mean syllable bandwidth (Hz)	-0.407	-0.057
Mean note minimum frequency (Hz)	-0.085	0.679
Mean note bandwidth (Hz)	-0.313	-0.222
Mean note 5% frequency (Hz)	-0.216	0.612
Mean note 90% bandwidth (Hz)	-0.233	-0.285

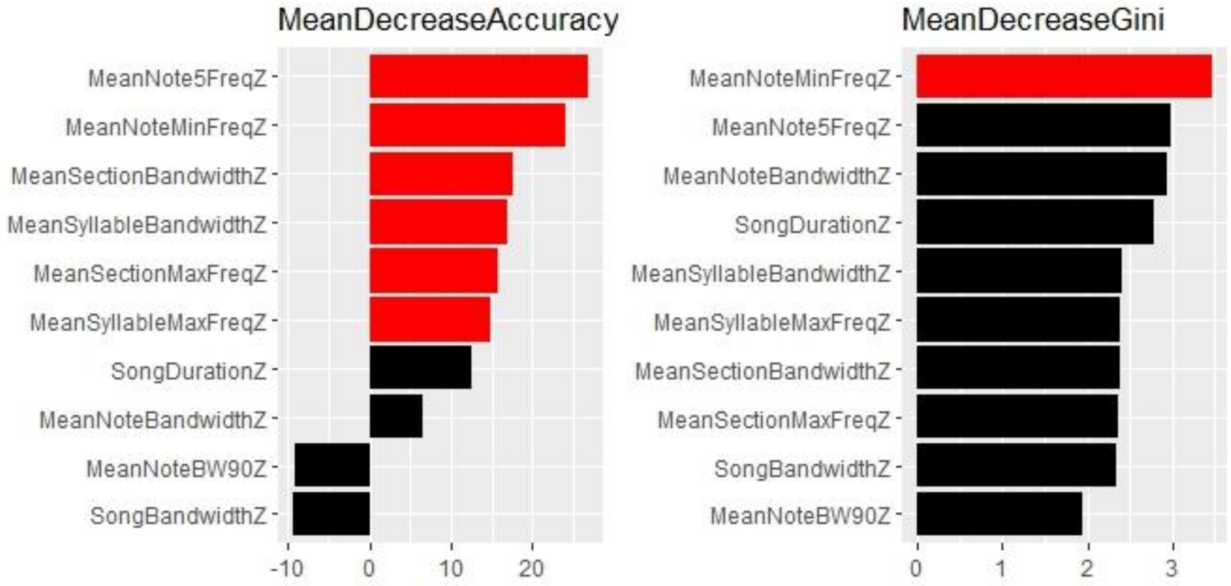

FIGURE S7. Relative importance and significance of 10 acoustic variables (standardized to z-scores) for correctly classifying 195 short songs of 183 male Brewer's Sparrows recorded at sagebrush and alpine sites in western Colorado, May-July 2021, as measured by the mean decrease in accuracy (MeanDecreaseAccuracy) and the mean decrease in the Gini index (MeanDecreaseGini) in random forest analysis. Variables in red were identified as significant for classifying songs by site type in the *rfPermute* package in R. Mean decrease in accuracy and mean decrease in Gini index metrics are defined in Methods. "Z" indicates that variables were standardized to a Z-scale for analysis.

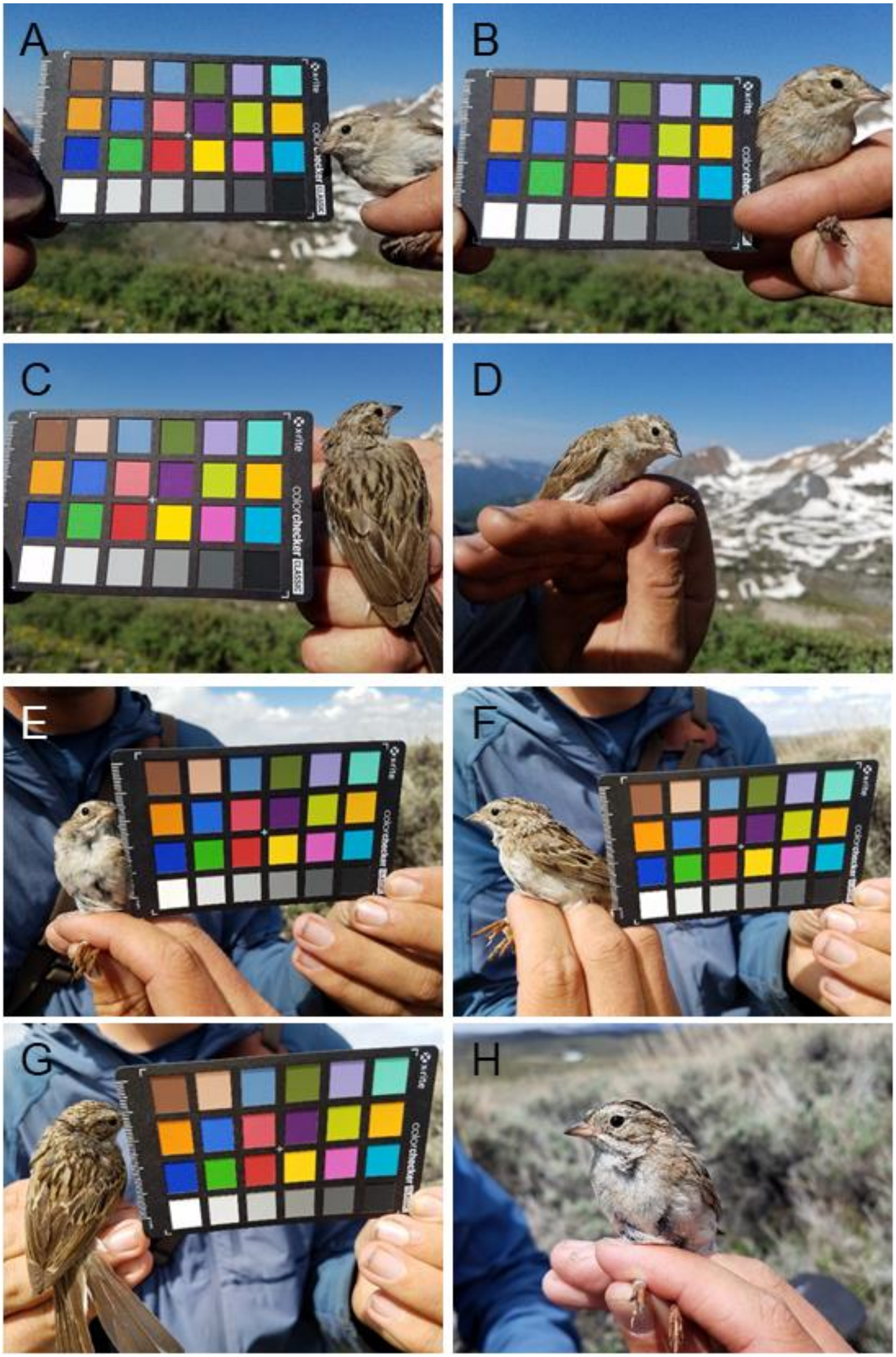

FIGURE S8. Digital photographs of male Brewer's Sparrows at an alpine site (Scarp Ridge; A-D) and a sagebrush site (Muddy Creek Road; E-H) in western Colorado.

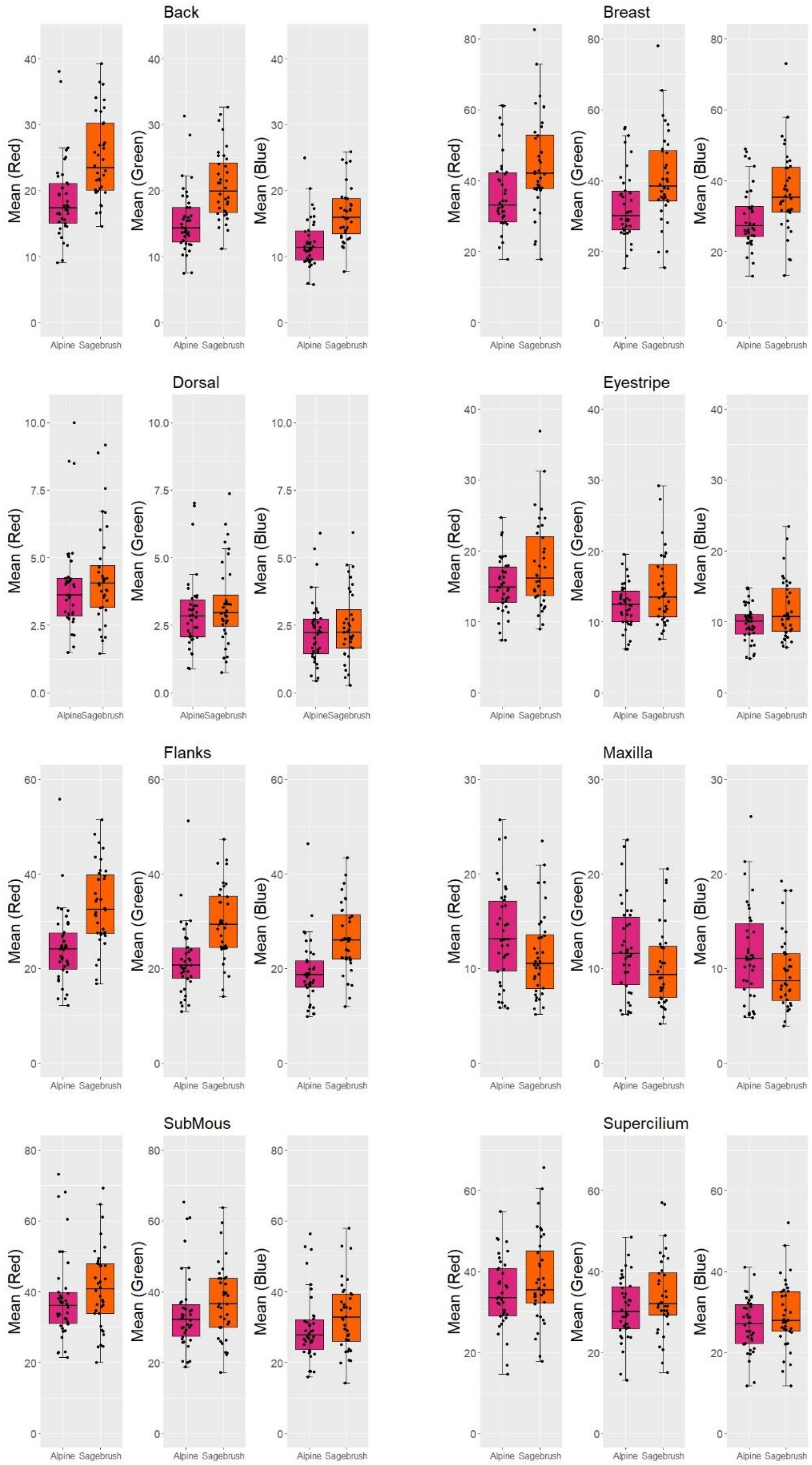

FIGURE S9. Jittered boxplots showing medians and interquartile ranges for linear, normalized, mean RGB reflectance values of eight features of male Brewer's Sparrows at alpine (pink; n = 38 males) and sagebrush (orange; n = 36 males) sites in western Colorado, 2021.