Dialect differences correlate with environment in migratory coastal White-crowned Sparrows

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ABSTRACT

Vocalization behavior in birds, especially songs, strongly affects reproduction, with differences in vocalizations between populations often interpreted as evidence of divergence. Previous work has demonstrated that the White-crowned Sparrow (*Zonotrichia leucophrys*) has multiple dialects, including between neighboring (and genetically distinct) subspecies *nuttalli* and *pugetensis*. However, it is unknown whether the divergence in their songs correlates to environmental or geographical factors. Previous work has relied on limited amounts of data because traditional methods to study bird songs rely on the manual annotation of song spectrograms into individual syllables. Here we explore the performance of automated machine learning methods of song annotation, which can process large datasets more efficiently, paying particular attention to the question of subspecies differences. We utilize a recently published artificial neural network to automatically annotate hundreds of White-crowned Sparrow vocalizations across two subspecies. By analyzing differences in syllable usage and composition, we find that *nuttalli* and *pugetensis* have significantly different dialects. Our results are consistent with the interpretation that these differences are caused by the reduction in the number of shared syllables in the White-crowned Sparrow repertoire, as reported by some previous research. Our large sample size enables us to demonstrate that divergence in dialects is correlated with environmental difference, but not with geographical distance, and is also associated with migratory status. Our findings support the hypothesis that the evolution of vocalization behavior is affected by both biotic and abiotic environments, in addition to population structure.

Keywords: Bird songs; Bioacoustics; Machine learning; *Zonotrichia leucophrys*.

LAY SUMMARY

- Bird song is an important behavior because it is important in bird communication and reproduction.

- White-crowned Sparrows in western North America are known to use different songs along their range, but it is unknown if those songs vary due to the environment.
- We used machine learning to analyze these songs and found that populations of White-crowned Sparrows can be differentiated based on their song dialects.
- Environmental factors during the summer months exert a greater influence on song evolution in migratory subspecies.

INTRODUCTION

Vocalization is the primary method of bird communication (Catchpole and Slater 2008, Brumm and Zollinger 2013). Unlike other sensory communication channels (e.g., visual, chemical, tactile), vocalizations can transmit long distances and have a fast rate of evolutionary change, due in part to cultural transmission (Slater 1986, Slater 1989, Slabbekoorn and Smith 2002a, Podos and Warren 2007, Derryberry 2009; but see Mason et al. 2017). In birds, vocalization behavior can be distinguished into two types: calls and songs (Catchpole and Slater 2008). Bird calls are short and simple notes that are produced by both sexes year-round and are usually used for alarms and within-flock contact (Evans et al 1993, Marler 2004, Kondo and Watanabe 2009). In contrast to calls, bird songs are more complex and melodious. While it was previously thought that songs were mostly produced by males during the breeding seasons, with common functions of territory defense and mating (Kroodsma and Byers 1991), more recent observations and research shows that females also produce songs, and songs are being produced also in non-breeding contexts (DeWolfe et al 1989, Baptista et al 1993, DeWolfe and Baptista 1995, Odom et al 2014, Webb et al 2016, Riebel et al 2019).

Song evolution is influenced by biotic and abiotic factors. Vocal behaviors vary among species and play a role in species recognition (Beer 1971, Emlen 1972, Baker et al. 1981b, Freeberg et al 1995). Even closely related populations can have different songs (i.e., dialects) that they can use to tell each other apart (Cunningham and Baker 1983, Thompson et al. 1993, MacDougall-Shackleton 2001). Many taxa are functionally reproductively isolated due to song differentiation (e.g., Provost et al. 2021), potentially contributing to speciation of distinct populations. These differences can also arise due to evolution of other traits like body size and beak shape, which indirectly influence the sounds a bird can produce (Nowicki et al. 1992; Podos and Nowicki 2004a, 2004b, Derryberry 2009). Within species, sexual selection via same-sex competition and mate choice can lead to multiple different outcomes (reviewed by Searcy and Andersson 1986, Nowicki et al 1998). Another biotic factor influencing song is song learning, which is present in three orders of birds (Apodiformes, Psittaciformes, and Passeriformes). In the aptly named oscine songbirds (Order: Passeriformes, Suborder: Passeri), songs are learned by juvenile individuals listening to the singing adults immediately around them. As such, geographically restricted song variants emerge through time (Podos and Warren 2007), resulting in a pattern analogous to isolation-by-distance (Nelson et al 2004). Rarely, older individuals can learn new variants, for example mimics (Robinson 1974) and brood parasites (Rothstein and Fleischer 1987, Warren 2002)

In contrast to these biotic factors, the abiotic environment can impact the evolution of song by influencing the efficacy of its transmission. Lower frequencies and slower notes transmit more effectively through denser vegetation, and birds living in those habitats have songs with those characteristics (Slabbekoorn and Smith 2002a, Naguib 2003, Kopuchian et al. 2004, Seddon 2005, Baker 2006, van Dongen and Mulder 2006, Derryberry 2009). Likewise, birds in urbanized habitats sing at higher frequencies to prevent overlap with low-frequency

anthropogenic noise (Derryberry et al. 2016, Derryberry et al. 2020). In sum, differences in song between bird taxa can result from any combination of these biotic and abiotic factors.

White-crowned Sparrow

One well-studied songbird is the White-crowned Sparrow (*Zonotrichia leucophrys*), which has multiple dialects across North America (Baptista 1989). This species is known to sing year-round, not just during the breeding season, making them good candidates for understanding environmental impacts on their sounds (Blanchard 1941, Baptista 1974, Brenowitz et al. 1998). Non-breeding singing in *pugetensis* appears to be associated with flock hierarchy formation as well as hormone stimulation (DeWolfe and Baptista 1995), in contrast with *nuttalli* which sings due to a protracted breeding season.

They are known to have divergent dialects across multiple different populations, particularly at the subspecies level (Petrinovich and Patterson 1981, Baker et al. 1984, Lien and Corbin 1990). We focus here on *Zonotrichia leucophrys nuttalli* and *Z. l. pugetensis* (hereafter *nuttalli* and *pugetensis*, respectively). These subspecies have divergent dialects and population genetic differentiation (Lipshutz et al. 2017), suggesting that they are distinct evolutionary units. In addition, previous estimates show that dialects present in *pugetensis* are more wide-ranging than in *nuttalli* (DeWolfe and Baptista 1995). Songs help to identify these two subspecies, both for researchers and for the birds themselves. The dialects of these birds vary such that the subspecies can tell each other apart, suggesting that reproductive isolation is evolving. The subspecies have an area of hybridization in Mendocino County, CA, where intermediate individuals are found (Lipshutz et al 2016). Generally, subspecies *nuttalli* (Figure 1A) and *pugetensis* (Figure 1B) differ in the length of introductory whistle notes, presence of buzz notes, and length/presence of terminal trill notes (Dunn et al. 1995; Baptista and King 1980), though variation exists within populations and through time. Song is directly implicated in reducing gene flow between these populations, which have been at least partially genetically isolated for ~45,000 years (Lipshutz et al 2016). The different dialects of both *nuttalli* and *pugetensis* also show a geographic pattern within the subspecies, which are proposed to have been formed by dialect formation, either limited or biased dispersal, morphological variation, and/or habitat (Marler and Tamura 1962, 1964, Barker and Mewaldt 1978, Nelson 2000, Nelson et al. 2004, Kroodsma et al. 1985, reviewed by Podos et al. 2007, Derryberry 2009).

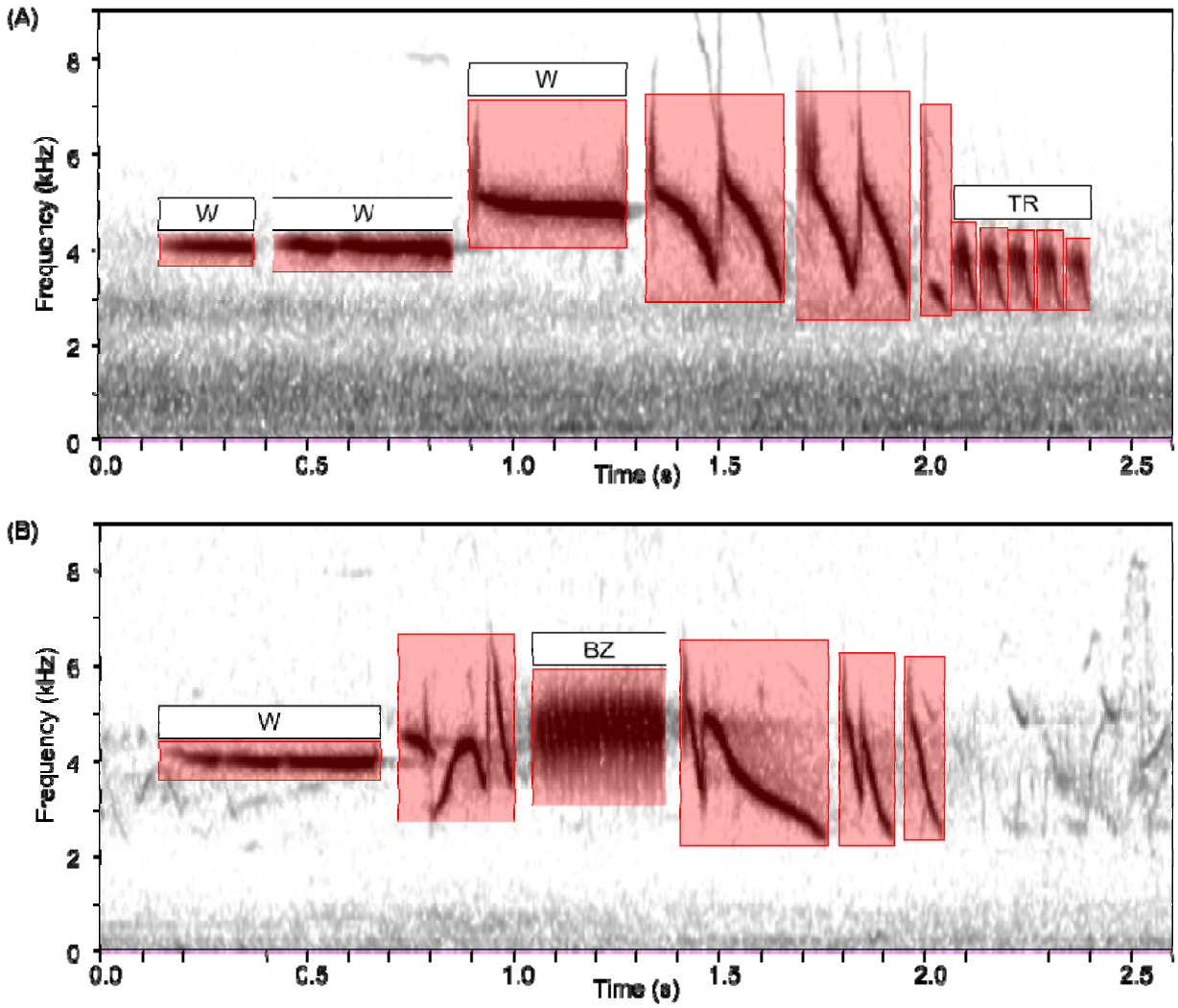

Figure 1. Spectrogram of (A) *nuttalli*, BLB11552, and (B) *pugetensis*, BLB07718, songs with manually annotation in red boxes. The X axis shows the duration time, in seconds, and the Y axes shows the frequency, in kilohertz. Higher frequencies represent higher pitch; and darker color represents higher amplitude i.e., louder sound. Each red box represents one annotated syllable, with the width representing the duration while the height indicates the differences of maximum and minimum frequency i.e., bandwidth. Note that background sounds are not annotated. Above certain notes are annotated the note type: W = whistle, BZ = buzz, and TR = trill of simple notes.

Though the two subspecies are phenotypically similar, with only slight differences in body size (Dunn et al. 1995), they do vary in their migratory behavior and geographic distributions. Subspecies *nuttalli* is a non-migratory subspecies that is distributed in coastal California (from ~40.4 to 34.3 degrees latitude; Banks 1963; Figure 2). In contrast, *pugetensis* is a migratory subspecies that is distributed from British Columbia to northwest California during summer, both coastal and inland, and expands to the southern part of its range through southern California during winter (Banks 1963, Patten et al. 2003, Baptista 1974, Heinemann 1981; Figure 2). Subspecies *pugetensis* thus shares its wintering grounds with three other subspecies: *nuttalli*, which is phenotypically similar, as well as *oriantha* and *gambelii*, which are distinguished by

differences in color of the beak and the lores (Welke et al 2021, Lisovski et al 2019). Moreover, *pugetensis* has clinal dialects along its range during the breeding season, with populations having the same dialect tending to migrate to the same location in the non-breeding season (Nelson 2000, Nelson et al 2004, Baptista 1974, Heinemann 1981) despite those locations not being segregated by song dialect (DeWolfe and Baptista 1995).

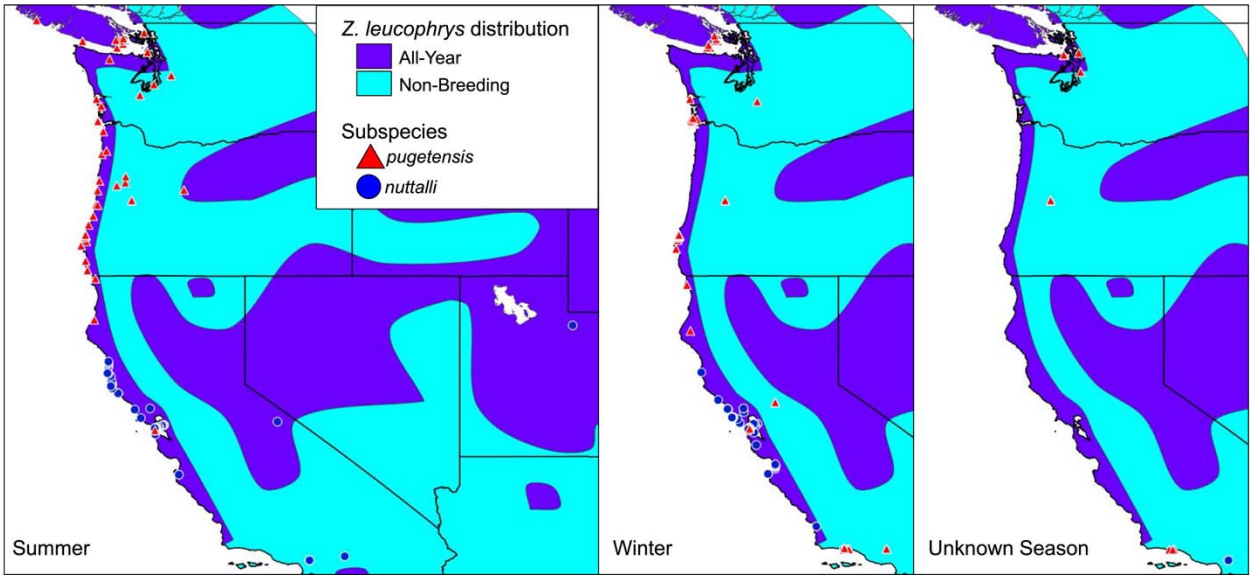

Figure 2. The geographical range of *Zonotrichia leucophrys* recordings used in this study. The sedentary *nuttalli* individuals are blue circles and the migratory *pugetensis* individuals are red triangles. Individuals in western California and Utah were outliers (see main text). Map below shows species distribution in all year (purple) and non-breeding season (light blue) (BirdLife International and Handbook of the Birds of the World 2021; Welke et al. 2021, Weckstein et al. 2001, Grinnell and Miller 1944, Taylor et al 2021, Cortopassi and Mewaldt 1965). Note that this map includes distributions for other subspecies (*gambelii* and *oriantha*). Individuals are separated by Summer (May to August, left), Winter (September to April, center) and season unknown (right).

Though there is compelling evidence that the divergence of White-crowned Sparrow songs is caused by sexual selection, there is less evidence on whether the evolution of vocalization behavior is correlated to the abiotic environment, such as climate, or geographic distance as a proxy for cultural evolution. Distinct differences in the histories of these two subspecies may be mediating environmental adaptation. Traditionally, *pugetensis'* breeding and wintering grounds were thought to be distinct, with the breeding not occurring south of Oregon and wintering only occurring in California (DeWolfe and Baptista 1995). However, *pugetensis* experienced a range shift east through the Cascade Mountains and North into Alaska because of habitat change and logging regimes (Welke et al 2021, Hunn and Beaudette 2014, Gibson et al 2013), and birds identified as *pugetensis* are found north of their supposed breeding grounds during the winter, though typically the reverse is not true (Sullivan et al, 2009; GBIF 2023). As such, it is likely that these shifts in location may be due to *pugetensis* being sensitive to environmental changes. In this paper, we use these White-crowned Sparrow subspecies to investigate whether the divergence in songs within and between subspecies is correlated to these abiotic factors. We

hypothesize that the different dialects are correlated to climates and geographical differences between the two subspecies. We also hypothesize that *pugetensis* may have a greater impact of environment on their songs compared to *nuttalli*, and that impact should be more apparent during the summer months.

METHODS

All work was done on MacBook Pro running macOS Ventura (13.0) with an 8 core Apple M1 chip and 8 GB of RAM.

Recording data and metadata

We downloaded our sound data from two sources: the Borror Laboratory of Bioacoustics (BLB; blb.osu.edu) directly from the database and the Xeno-Canto database (X-C; xeno-canto.org) by using *query_xc* function in the *warbleR* package version 1.1.26 (Araya-Salas and Smith-Vidaurre 2017) in R version 4.0.3 (R Core Team 2020). We used all recordings of *Z. l. pugetensis* and *Z. l. nuttalli* in the BLB. On X-C, we restricted recordings to be graded as Quality "A." We transformed the X-C recordings from MP3 format to WAV format by using the *readMP3* function in the *tuneR* package version 1.3.3 (Ligges et al. 2018) in R. We also converted recordings to have a sample rate of 48,000 Hz if needed using the *resamp* function in the *seewave* package version 2.2.0 (Suer et al 2008) in R. This resulted in 315 *nuttalli* recordings and 360 *pugetensis* recordings from BLB, plus four *nuttalli* recordings and 38 *pugetensis* recordings from X-C. Recordings were made between 1965 and 2005 and included metadata subspecies identification, date, locality, and recordist.

Both databases contained recordings from the region of interest that were not identified to subspecies; we assigned them using latitude as a criterion for breeding-season birds. The subspecies identification followed Lipshutz et al (2017). We assigned all summertime "unknown" individuals north of 40.544 and south of 48.461 to be *pugetensis*, and all summertime "unknown" individuals south of 38.419 and north of 37.803 to be *nuttalli*, because they do not have overlapping distribution within these regions during summer. Based on the distribution, we reassigned some mis-identified birds in the original datasets. We did not assign "unknown" individuals between the two regions because the two subspecies intergrade in that area. Moreover, "unknown" individuals that were further than ~110km (one decimal degree) from the coast were not classified as either subspecies as they likely belonged to subspecies *oriantha* or *gambelii*. For non-breeding seasons birds, we did not assign "unknown" individuals due to substantial amounts of overlap between *nuttalli*, *pugetensis*, *gambelii*, and *oriantha* which all overwinter through the region.

Our recordings were mostly restricted to the west coast of the United States and Canada. However, there were two localities that were further inland and at higher elevation (above 1900 m). As these localities were likely to influence our environmental analyses, we removed them from our downstream analyses (but see Supplemental). Our final dataset included 238 recordings from summer (111 *pugetensis*, 127 *nuttalli*), 461 recordings from winter (276 *pugetensis*, 185 *nuttalli*), and 16 with no date (11 *pugetensis*, 5 *nuttalli*) for a total of 715.

Manual Annotation

We followed the methodology of Provost et al. (2022) to annotate our samples. 201 (28%) of these samples were annotated for that study. For manual annotation, we input the recording to Raven Pro version 1.6.1 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab

of Ornithology 2019) and generated a spectrogram using a Fast Fourier Transform size of 512 samples. We annotated the spectrogram by drawing boxes that show the maximum and minimum values of the frequency and time of each syllable. All selections were output as TXT files. We then split the original recordings and their corresponding annotation files into subsets that had approximately equal amounts of annotated syllables and silence using previously generated scripts (Provost et al. 2022). Although TweetyNet can identify and segment multiple kinds of syllables, we annotated all syllables as "1".

Using Machine Learning for Bioacoustics

Bird songs have been studied for a long time with many methods for qualitatively and quantitatively describing their sounds having been developed. Traditional methods require researchers to analyze and measure song parameters manually. Since the mid 20th century, the most popular methods of analysis use spectrograms, which display frequencies through time (Borror and Reese 1953; Figure 1). In the digital era, these spectrograms are generated digitally by using fast Fourier transforms (e.g., via Raven; K. Lisa Yang Center for Conservation Bioacoustics Cooley and Tukey 1965). Many analyses require every individual syllable or note on the spectrogram to be identified and annotated, or segmented, for downstream processing. Segmentation is time intensive and the amount of data that can be processed is limited (Swiston et al. 2009).

Recently, machine learning and artificial intelligence techniques have been used broadly in biological science (e.g., Pelletier and Carstens 2018, Cushman et al 2017, Viener et al 2022, Schrider and Kern 2017, Buxton et al 2018, Clapham et al 2020). Machine learning has also been applied to syllable segmentation with high accuracy via the neural network TweetyNet (Cohen et al. 2020). Originally trained on single species, the algorithm has since been applied to a suite of birds (Provost et al 2022) with similarly high performance, allowing for the use of larger datasets. Although the segmentation itself has been sped up, it is unknown whether this and other automatic segmentation methods effectively recapitulate known differences in populations. Here, we examine whether we can apply automatic syllable recognition to the different dialects of White-crowned Sparrow songs.

To automatically annotate the White-crowned Sparrow songs, we used the TweetyNet model trained on *Zonotrichia leucophrys* from Provost et al. (2022). The latter analyses included examples from all five subspecies. We then fine-tuned this model by continuing to train it on just data from *nuttalli* and *pugetensis*. We used vak version 0.6.0 and TweetyNet version 0.8.0 for that and we used identical parameters to (Provost et al. 2022). After being fine-tuned, we had the model predict the locations of syllables for all data, including data that TweetyNet had never seen before. We assessed model performance (syllable error rates, accuracy, precision, recall, and f-score) for each subspecies.

Summary statistics

Once we had syllables segmented for all recordings, we extracted bioacoustic data for each annotation. Data were calculated using the *spectro_analysis* function in the *warbleR* package in R. The data extracted from syllables include bandwidth (the difference between highest frequency and lowest frequency of the syllable), time duration (the length of the syllable), center frequency (central frequency between the upper and lower cutoff frequencies), average slope (slope of the change in dominant frequency), and inflection points (the local extrema). For each recording, we calculated the centroid value across all syllables. We then calculated a distance

between centroids between all individuals within and between subspecies, which we used as a representation of song distance.

Principal Components Analysis and ANOVAs

We performed a principal components analysis (PCA) on bandwidth, time duration, center frequency, average slope, and inflection points of syllables. To do this, we used the *prcomp* function in the *stats* package version 4.1.2 in R. We centered and scaled all data. The PCA results were used to compare differences between the subspecies. We used an ANOVA on both the raw statistics and results of PCA to test the differences between the two subspecies and two seasons (winter and summer) using the *aov* function in the *stats* package in R. We analyzed significance for this and all other analyses using an alpha value of 0.05.

Multiple Matrix Regression with Randomization

To determine whether differences in songs were being driven by differences in either the abiotic environment or geographic distance, we used multiple matrix regression with randomization analysis (MMRR) in R (Wang 2020). This technique allows one to correct correlations between environmental features and geography. To calculate geographic distances as a proxy for cultural evolution, we calculated the Euclidean distance between individuals with respect to latitude and longitude. For environmental differences, we downloaded 19 climate variables from WorldClim (Fick and Hijmans 2017) and extracted values at each locality in our dataset. We then calculated Euclidean distance between individuals with respect to those values. MMRR models were created such that we predicted song divergence from geographic distances and environmental differences simultaneously (i.e., song divergence ~ geographic distance + environmental distance). Note that there were several *nuttalli* individuals across these analyses for whom either geographic or environmental information was lacking; as such, our dataset for the MMRR analyses included 398 *pugetensis* and 317 *nuttalli*.

Because *pugetensis* is a subspecies that migrates through the year, we separated our data by seasons: summer (May to August, when *pugetensis* are in their breeding range) and winter (September to April, when *pugetensis* are in their non-breeding range; Chilton et al. 2020). We performed these MMRR analyses described above with only the winter and summer datasets separately because the different ranges due to migration of *pugetensis* may result in variation in geographical distance and ecological distance.

RESULTS

First, we analyzed how the machine learning model performed in syllable annotation. These models had high performance across both subspecies. The syllable error rate of our trained machine learning model's annotations was 15% for *nuttalli* and 4% for *pugetensis* (Table 1), such that for both subspecies, the model predicted more syllables than were present. Accuracy, precision, recall, and F-score were all above 0.91 for both subspecies, with *nuttalli* having 2–3% better performance than *pugetensis* (Table 1).

Table 1. The machine learning model showed a high accuracy in annotating the syllables of both *pugetensis* and *nuttalli* for all data.

Subspecies	Syllable Error Rate	Accuracy	Precision	Recall	F-score
nuttalli	15%	0.96	0.96	0.93	0.95
pugetensis	4%	0.93	0.94	0.91	0.92

PCA

We found that our PCA on syllable variables explained a high proportion of the data, with the five PCs explaining 45%, 20%, 17%, 13%, and 3% of variation, respectively (Table 2). Syllables with high PC1 had wide bandwidth, short time, low frequency, few inflection points, and negative slopes (Table 2). Subspecies *nuttalli* and *pugetensis* used different dialects and songs were different across seasons (Table 3). Subspecies varied in PC1 (mean *pugetensis* = -0.16, mean *nuttalli* = 0.21) and PC2 (mean *pugetensis* = 0.25, mean *nuttalli* = -0.32) reflecting significant differences in center frequency (mean *pugetensis* = 3.12, mean *nuttalli* = 2.92), inflection points (mean *pugetensis* = 13.13, mean *nuttalli* = 10.85), and slope (mean *pugetensis* = -1.06, mean *nuttalli* = -18.35). Across seasons, songs varied in PC1 (mean winter = 0.13, mean summer = -0.23, mean unknown = -0.36) and PC4 (mean winter = 0.03, mean summer = -0.10, mean unknown = 0.41), reflecting differences in duration (mean winter = 0.16, mean summer = 0.18, mean unknown = 0.16) and inflection points (mean winter = 11.50, mean summer = 13.26, mean unknown = 13.07). PC3, PC5, and bandwidth were not different across season or subspecies.

Table 2: Principal component importance and rotation of individual PC axes for song across five summary statistics of song properties.

		PC1	PC2	PC3	PC4	PC5
Importance	Standard deviation	1.50	1.01	0.92	0.80	0.43
	Proportion of variance	0.45	0.20	0.17	0.13	0.03
	Cumulative proportion	0.45	0.65	0.83	0.96	1.00
Rotation	Bandwidth	0.37	0.34	0.65	-0.55	0.12
	Duration	-0.52	-0.05	-0.15	-0.68	-0.48
	Center	-0.42	-0.07	0.7	0.42	-0.35
	Inflection	-0.61	0.08	0.07	-0.09	0.77
	Slope	-0.14	0.93	-0.19	0.2	-0.17

Table 3: Differences between season (winter-summer) and subspecies (*pugetensis-nuttalli*) using ANOVAs while controlling for both. Df = 1 for all comparisons. Note that unknown season individuals were removed. SSQ = sum of squares. MSQ = mean sum of squares. F = F-statistic. P = p-value.

		Winter-Summer				*pugetensis-nuttalli*			
		SSQ	MSQ	F	P	SSQ	MSQ	F	P
Raw	Bandwidth	0.1	0.091	1.08	0.74	1.4	1.36	1.61	0.20
	Center	1.7	1.715	2.52	0.11	7.2	7.17	10.54	**0.00***
	Duration	0.094	0.093	13.12	**0.00***	0.003	0.00	0.45	0.49
	Inflection	482	482.3	10.08	**0.00***	865	865	18.09	**0.00***
	Slope	167	167	0.207	0.64	53005	5305	65.57	**0.00***
PCA	PC1	21.1	21.1	9.8	**0.00***	24.1	41.1	11.30	**0.00***
	PC2	0.1	0.06	0.059	0.80	59.8	59.8	63.4	**0.00***
	PC3	0.3	0.26	0.3	0.58	3.2	3.18	3.74	0.05
	PC4	3	3.02	4.73	**0.03***	2	1.97	3.07	0.07
	PC5	0	0	0	0.99	0.25	0.25	1.33	0.24

MMRR

We found that year-round, environmental (climate) distance explained song divergence when looking at both subspecies together, but geographic distance did not ($R^2 = 0.01$, climate $p = 0.02$, geography $p = 0.69$; Table 4). Neither climate nor geography significantly explained song divergence in winter alone ($R^2 = 0.00$, climate $p = 0.13$, geography $p = 0.13$) or summer alone ($R^2 = 0.00$, climate $p = 0.75$, geography $p = 0.68$) for both subspecies together. Subspecies *nuttalli* showed no relationship between song divergence and climate or geography for any season (climate $p > 0.20$, geography $p > 0.26$). In contrast, subspecies *pugetensis* showed significant impact of geography year-round ($R^2 = 0.03$, climate $p = 0.00$, geography $p = 0.23$) and both geography and climate in summer only ($R^2 = 0.14$, climate $p = 0.01$, geography $p = 0.02$). There was no relationship in winter alone ($R^2 = 0.00$, climate $p = 0.64$, geography $p = 0.52$). These patterns cannot be explained solely by differences in year recorded (see Supplemental).

Table 4: MMRR analysis shows that divergence in songs of *pugetensis* and *nuttalli* is strongly correlated with environmental differences.

					T-values		Coefficients		P-Values		
Subspecies	Season	N	R^2	F	Geo.	Env.	Geo.	Env.	Geo.	Env.	Overall
Both	Year	694	0.01	980	3.48	25.22	0.01	0.08	0.69	**0.02***	**0.00***
	Winter	456	0.00	92.5	-13.13	12.89	-0.07	0.07	0.13	0.13	0.18
	Summer	234	0.00	3.83	2.77	-2.02	0.02	-0.02	0.68	0.75	0.91
N	Year	305	0.00	61.7	-4.88	10.60	-0.03	0.07	0.60	0.20	0.44
	Winter	181	0.01	82.8	6.65	1.31	0.09	0.02	0.28	0.85	0.13
	Summer	124	0.00	15.7	5.48	-5.11	0.11	-0.10	0.26	0.25	0.43
P	Year	389	0.03	1207	37.46	-9.94	0.22	-0.06	**0.00***	0.23	**0.00***
	Winter	275	0.00	12.2	-4.95	3.72	-0.04	0.03	0.52	0.64	0.82
	Summer	110	0.14	504	-20.67	30.96	-0.42	0.62	**0.02***	**0.01***	**0.02***

DISCUSSION

Our analysis demonstrates that the trained machine learning model by TweetyNet has high accuracy with low error rates and can annotate *nuttalli* and *pugetensis* songs. Our results suggest that the annotation by the machine learning model is as reliable as manual annotations and produces comparable data. Given its computational efficiency, machine learning can be a useful tool to automatically annotate bird songs and extract data because it can be used to process large datasets quickly and accurately.

We found significant differences in the syllables used in the songs of *nuttalli* and the songs of *pugetensis*, which corroborates previous evidence that these two taxa have divergent dialects (e.g., Lipshutz et al. 2017). Our bioacoustic metrics found that on average, *nuttalli* syllables were lower in pitch, had fewer inflections, and had more negative slopes compared to *pugetensis*. At present our analyses cannot distinguish whether this is due to subspecies using different syllables or the same syllables at different frequencies. Preliminary analyses of syllable shape suggest that the differences are mediated by use of different syllables (KLP pers. comm.) but further investigation is needed to confirm.

We also found significant differences between the seasons. Syllables used in summer were on average longer and had more inflection points, suggesting higher complexity. Although more complex songs have been found to be sexually selected for in other species (e.g., Catchpole 1987), complexity itself is likely a result of many competing selective pressures within and between species including migratory behavior (Read and Weary 1992). In *Zonotrichia leucophrys*, different parts of the song appear to have different function (Nelson and Poesel 2007): introductory whistles (as well as buzzes) are highly conserved and serve as species identifiers, terminal trills are moderately conserved and indicate dialect, and other notes in the song are highly variable and indicate individual identity. At the same time, this species is known to modulate its song with respect to the environment, being especially impacted by noise transmission (Derryberry et al 2009, Luther et al 2016, Derryberry et al 2020). Our findings indicate that White-crowned Sparrows use different aspects of their repertoire for different functions during the breeding and non-breeding seasons.

The differences in dialect between subspecies and seasons are compounded by the fact that the two subspecies have different responses to the environment, and that these are dependent on the time of year analyzed. The migratory *pugetensis* shows a high impact of environment and geographic distance on songs recorded in summer, but not winter. Year-round, geographic distances remain significant predictors of song divergence in this subspecies, suggesting that winter and summer songs may be different. In contrast, the sedentary *nuttalli* shows no relationships between environment or geographic distance with respect to song. The evidence suggests that overall, the migratory subspecies *pugetensis* shows higher impact of environment during the breeding season. This may be driving the patterns where both species are combined in those seasons; alternatively, the two subspecies may still be adapted to their relatively different environments. It is likely that these factors (i.e., migration and seasonality) have contributed to the evolution of dialect differences.

We performed a *post-hoc* analysis of the environments that each of the subspecies occupy during different seasons (see Supplemental) and found that these environments are significantly different based on climate data. Year-round, *nuttalli* occupies warmer and drier areas than *pugetensis* does in the summer. In contrast, wintering *pugetensis* experience nearly the full yearly range of temperature and precipitation across both subspecies, and overall experience more variable and more seasonal climates than *nuttalli*. This evidence indicates that *pugetensis* has a higher sensitivity to the environment. A synthesis across multiple birds hypothesizes that migration occurs as a mechanism to avoid harsh winters and exploit resource surpluses (Somerville et al 2015, Global Ecology and Biogeography); given that the range of *pugetensis* is wide-spread and has been changing in response to habitat disruption (Welke et al 2021, Hunn and Beaudette 2014, Gibson et al 2013), we hypothesize that *pugetensis* as a subspecies may be more sensitive to fluctuations in habitat and climate, leading to more sensitivity in their songs. In addition, because *pugetensis* is known to have dialects ranging over larger areas that encapsulate more variable climates (DeWolfe and Baptista, 1995), it is possible that the selection pressures to adapt song to environment is higher across the range of this subspecies.

Caveats

Several caveats are worth documenting to place our results in the appropriate context. All analyses shows that geographic distance is not correlated to the divergence in song across these two subspecies, which is not consistent with our hypothesis. Some explanations are as follows. Firstly, although the number of recordings of *pugetensis* in non-breeding habitats is large, they do not cover the entire projected breeding range. Second, since our recordings are all from the BLB and X-C database from 1965 to 2005, we do not know if songs have changed during this period and so we may have quite different songs in the same area. Over the time scale of the study, White-crowned Sparrows are known to have demonstrably changed their song dialects (e.g., Derryberry 2009). However, even if we control for the year of recording, we still find significant relationships with spatial factors like geography and environment (see Supplemental). Third, our analysis only used each syllable's characteristics but did not analyze the type, syntax, or order of syllables. It is possible that they used the same syllables but different orders in some dialects. Furthermore, because White-crowned Sparrows learn their songs not only directly from their parents but also from neighboring community (Cunningham and Baker 1983) that with same or similar songs, it is unknown that whether some juveniles learned songs from the other subspecies, especially in the hybrid zone, or some late-hatched *nuttalli* may have chance to expose to the environment that migrated *pugetensis* already arrived in their winter habitats. It is

hard to answer these questions with only the recordings and data of their locality and habitats. We need more research and specific data to solve the problems. However, the strong positive relationship between ecological differences and song dialects in winter corresponds with our original hypothesis. However, in this research, we only analyzed the numeric data of the features of bird songs and the climate of each locality, but we did not investigate the other properties of each habitat, such as the habitats' type. We still need further research on whether the abiotic environments directly or indirectly affect the evolution of bird songs and what is the mechanism of it.

In conclusion, using machine learning for automatic analyses, we evaluated divergence within and between two subspecies of the White-crowned Sparrow. Our results indicated that *Zonotrichia leucophrys pugetensis* and *Z. l. nuttalli* have significantly different songs from each other, and both sing different songs in breeding and non-breeding contexts. The divergence in dialects correlate with spatial factors such as geographic distance and climate, but subspecies identity and season mediate the response.

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