Narrow Contact of Desert Sage Sparrows (Amphispiza belli nevadensis and A. b. canescens) in Owens Valley, Eastern California: Evidence from Mitochondrial DNA, Morphology, and GIS-Based Niche Models

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Source: Ornithological Monographs No. 63

Published By: American Ornithological Society

URL: https://doi.org/10.2307/40166900

Ornithological Monographs
Volume (2007), No. 63, 78–95

CHAPTER 7

NARROW CONTACT OF DESERT SAGE SPARROWS (*AMPHISPIZA BELLI NEVADENSIS* AND *A. B. CANESCENS*) IN OWENS VALLEY, EASTERN CALIFORNIA: EVIDENCE FROM MITOCHONDRIAL DNA, MORPHOLOGY, AND GIS-BASED NICHE MODELS

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ABSTRACT.—We investigated the distribution of interior subspecies of Sage Sparrows (*Amphispiza belli nevadensis* and *A. b. canescens*) in Owens Valley, eastern California. Our primary goals were to establish the geographic limits of subspecies' nesting ranges in this region, to examine whether the two forms are in contact, and to elucidate their evolutionary history. Mitochondrial DNA (mtDNA) analysis and discriminant-function classification of size pointed to a narrow contact zone near Bishop, Inyo County, with evidence for limited sympatry and possible intergradation or hybridization. Estimates of mtDNA gene flow are low. The contact zone occurs toward the northern end of a bioclimatic gradient that spans the valley from the Great Basin in the north (cold and wet) to the Mojave Desert in the south (hot and dry). Ecological and bioclimatic data show slightly different patterns but generally suggest a transition that coincides with the distributional limits of the two forms. According to population genetic measures, both subspecies have expanded rapidly into Owens Valley. Because *A. b. nevadensis* and *A. b. canescens* occupy distinctive vegetation–climate associations, local adaptation to conditions in this region determines the extent to which the two forms can coexist at the margins of their respective ranges. *Received 30 July 2006, accepted 9 February 2007.*

RESUMEN.—En este trabajo, mostramos los resultados de una intensa investigación sobre la distribución de las subespecie de interior del Zacatonero de artemisa (*Amphispiza belli nevadensis* and *A. b. canescens*) en el valle de Owens, al este de California. El objetivo principal de nuestro estudio fue establecer los límites geográficos de los rangos de nidificación de las subespecies en la región, con el fin de determinar si ambas formas se encuentran en contacto, y así elucidar su historia evolutiva. Los análisis de ADN mitocondrial y una función discriminante para el tamaño de las aves revelaron una delgada zona de contacto cercana a Bishop, condado de Inyo, con evidencia de simpatría limitada y posible intergradación o hibridación. Las estimaciones del flujo génico para el mtDNA dan valores bajos. La zona de contacto ocurre en el límite norte del gradiente bioclimático que cubre el valle desde la gran cuenca hidrográfica en el norte (frío/ húmedo) hasta el desierto de Mojave en el sur (caluroso/seco). Los datos ecológicos y bioclimáticos muestran patrones levemente distintos pero, por lo general, sugieren una transición que coincide con los límites de distribución de las dos formas. De acuerdo con los parámetros poblacionales estudiados, las dos subespecies se han extendido rápidamente en el valle de Owens. Dado que *A. b. nevadensis* y *A. b. canescens* ocupan asociaciones diferentes de vegetación-clima, las adaptaciones locales a las condiciones de cada región determinan hasta qué punto las dos formas pueden coexistir en los márgenes de sus rangos respectivos.

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This is one of a series of papers aimed at understanding the systematic relationships of Sage Sparrow (*Amphispiza belli*) subspecies in western North America. Of the five taxa currently recognized (*A. b. belli*, *A. b. clementeae*, *A. b. cinerea*, *A. b. canescens*, and *A. b. nevadensis*; Martin and Carlson 1998), three (*A. b. belli*, *A. b. canescens*, and *A. b. nevadensis*; Fig. 1) have been the focus of ongoing investigations that began in 1977 with the collection of samples of specimens by N.K.J. for a study of morphometric and allozyme variation (Johnson and Marten 1992). In addition to the allozyme study, Johnson and Cicero (1991) used mitochondrial DNA (mtDNA) sequencing of a small fragment of the cytochrome-*b* gene to assess genetic relationships among the three subspecies. Over the past several years, we have extended this analysis to include significantly larger numbers of samples using both polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) and complete sequencing of cytochrome *b*, and to correlate genetic and phenotypic patterns with bioclimatic gradients using geographic information system (GIS) modeling (C. Cicero and N. K. Johnson unpubl. data). Most recently, Cicero and Johnson (2006) analyzed morphological variation between *A. b. canescens* and *A. b. nevadensis* to refute the claim (Patten and Unitt 2002) that these two forms are not diagnosable and should be synonymized. Finally, work in progress entails analysis of vocal variation in relation to morphological and molecular differences where *A. b. canescens* and *A. b. nevadensis* approach in Owens Valley, eastern California, (Mono and Inyo counties). This intensive investigation of potential contact in Owens Valley was the focus of one of N.K.J.'s final field trips in 2002.

Owens Valley stretches north–south for ~160 km and is bounded by the Sierra Nevada to the west and the White and Inyo mountains to the east. The mountains include the highest peak in the contiguous United States (Mount Whitney, at >4,400 m), and the valley floor is at 1,200 m, which makes it one of the deepest valleys in the United States. Owens Valley is also the westernmost "graben" in the Basin and Range Province, a downdropped block of land between two vertical faults. Desert shrub communities typical of the rain shadow of the Sierra Nevada dominate the vegetation of the valley floor. These plant associations grade sharply from the Great Basin in the north to the Mojave Desert in the south. The Owens River runs through the valley, providing important riparian and wetland habitat that has suffered significant losses because of water use over the past century.

Grinnell and Miller (1944) reported that the northern limit of *A. b. canescens*, which in California is a summer resident of desert scrub in the northern and western Mojave Desert and in the San Joaquin Valley, occurs in the vicinity of Benton in Benton Valley, Mono County. They proposed that it intergrades there with *A. b. nevadensis*, a significantly larger subspecies (Johnson and Marten 1992, Cicero and Johnson 2006) that breeds primarily in sagebrush (*Artemesia tridentata*) of the Great Basin region. On the basis of allozymes and morphology, however, Johnson and Marten (1992) firmly concluded that populations near the north end of the White Mountains (including Benton Valley as well as Chalfant Valley just to the south) are *A. b. nevadensis*. Although it is possible that the area of contact or intergradation has moved southward during the past 60 years, there is no evidence to support this hypothesis. Furthermore, if any change had occurred, one would expect a northward shift of the warmer-adapted *A. b. canescens* associated with increased summer temperatures (e.g., Johnson 1994).

From their findings, Johnson and Marten (1992:18) surmised that

> if a region of contact and intergradation exists between these forms it must be situated somewhere in the 100 mile [160 km] corridor between the southernmost certain breeding population of *A. b. nevadensis* (Chalfant Valley) and the northernmost definite nesting population of *A. b. canescens* (Coso Junction [see Fig. 1]). Investigation of this zone should yield information on the possible biologic species status of these two strongly differentiated forms.

To follow up on this earlier work, we used molecular, morphological, and bioclimatic data to examine distributional, evolutionary, and ecological patterns within *A. belli* in eastern California. Specifically, our goals were to (1) establish the nesting limits of *A. b. canescens* and *A. b. nevadensis* in or near the Owens Valley; (2) examine whether the two forms may come into contact and, if so, delineate the contact zone;

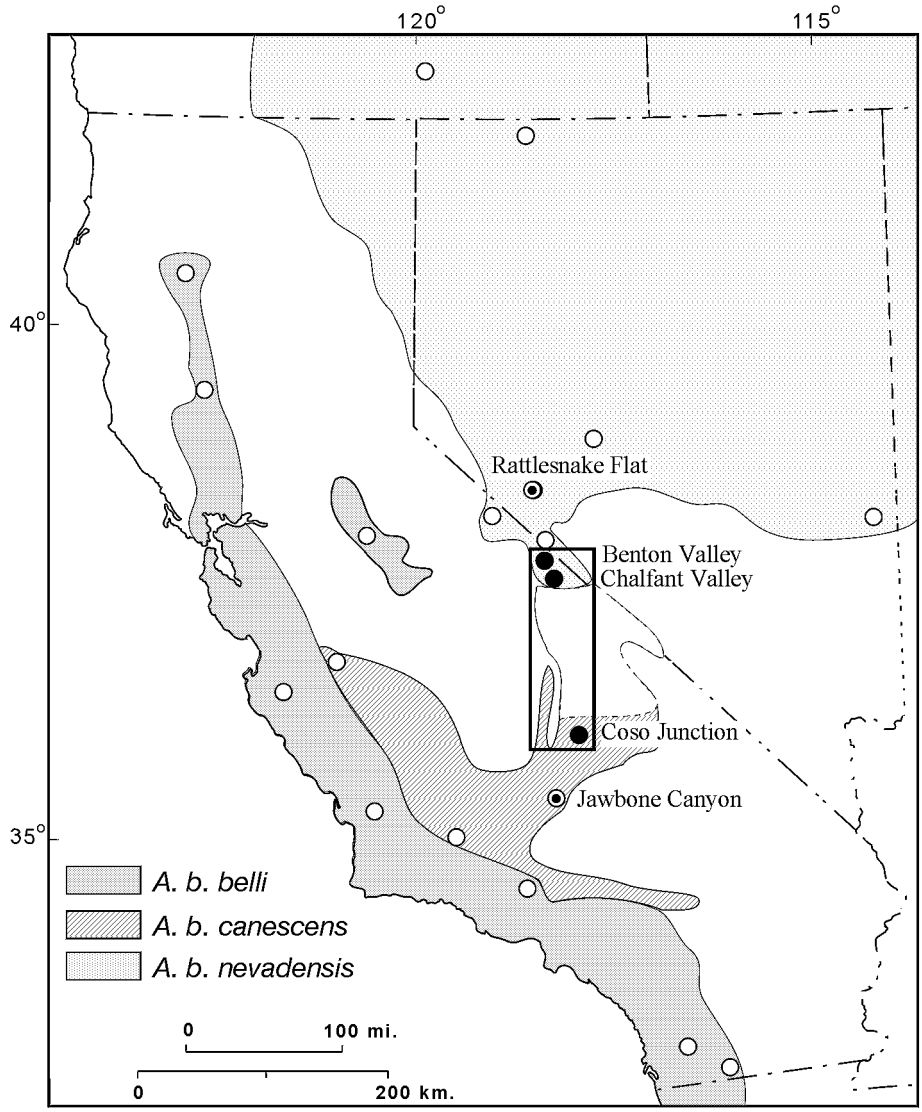

FIG. 1. Breeding distribution of three subspecies of *Amphispiza belli* in the far western United States. The box outlines the study area of potential contact between *A. b. nevadensis* and *A. b. canescens* in Owens Valley, eastern California. Circles show 21 of 22 samples analyzed previously by Johnson and Marten (1992); one sample is not shown because it contained postbreeding, upslope migrants. Open circles have been analyzed elsewhere for mtDNA (C. Cicero and N. K. Johnson unpubl. data) and were not included in the present study. Two samples (Rattlesnake Flat and Jawbone Canyon) were included as references outside the study area. Three samples (Benton Valley, Chalfant Valley, and Coso Junction) show definite nesting limits of the two forms before this study. Grinnell and Miller (1944) reported that specimens near Benton represent *A. b. canescens* or intergrade toward *A. b. nevadensis*, whereas Johnson and Marten (1992) concluded that populations at Benton and Chalfant valleys are definitely *A. b. nevadensis* (see text).

and (3) understand the evolutionary history of the species in this region. The results of the present study, together with additional mtDNA and nuclear analyses and a study of vocal variation in the Owens Valley (C. Cicero and N. K. Johnson unpubl. data), will confirm whether the two forms should be regarded as biological species. The taxonomic relationship of *A. b. nevadensis* and *A. b. canescens* to other subspecies, especially *A. b. belli*, will be discussed in a separate paper (C. Cicero and N. K. Johnson unpubl. data).

Materials and Methods

Field work and specimens examined.—N.K.J. began collecting specimens in the Owens Valley for this study in spring 1991, and subsequent field seasons often included a trip to the region for additional sampling (and trout fishing whenever possible). The last trip was taken by C.C. in 2004. During this period, we collected a total of 198 specimens with tissues, which supplemented existing samples (n = 357) taken for the broader analysis of allozymes and morphology (Johnson and Marten 1992). Specimens were prepared in the field as study skins, and tissues (heart, liver, muscle, kidney) were preserved in liquid nitrogen for later storage in ultra-low (–80°C) freezers. In general, our efforts focused on recording songs from breeding males and then targeting those same individuals for collection. Although detailed study of vocal variation will be presented elsewhere, the availability of vocal, morphological, and genetic data from the same birds provides a powerful data set for assessing biological species status. All specimens, tissues, and recordings are deposited in the collections of the Museum of Vertebrate Zoology, University of California, Berkeley (see Acknowledgments).

Samples from Owens Valley were grouped into 14 geographic areas for analysis, with two additional samples (Rattlesnake Flat, Jawbone Canyon) included as references from outside the area of potential contact (see Table 1, Fig. 2, and Appendix). Five of the 16 samples were studied previously by Johnson and Marten (1992), with supplemental collections at two locations (Benton Valley, Chalfant Valley) in 2002 and 2004. As noted above, Benton and Chalfant valleys represent the southernmost known nesting limit of *A. b. nevadensis*, and Coso Junction is the northernmost limit of known nesting for *A. b. canescens* (Johnson and Marten 1992).

Molecular analyses.—A broader geographic study (C. Cicero and N. K. Johnson unpubl. data) of cytochrome-*b* variation in 304 individuals of *A. b. belli*, *A. b. canescens*, and *A. b. nevadensis* (17 of 22 sample areas analyzed by

Table 1. Samples of *Amphispiza belli* analyzed for mtDNA (n) and morphology (adult males only) in and near Owens Valley. Distribution of distinct cytochrome-*b* haplotype groups (AC, AD, BC) is also indicated. See Figures 1 and 2 for sample locations.

	Sample name and number [a]	n [b]	Number of males [c]	AC	AD	BC
Ref	Rattlesnake Flat*	15 (2)	15			15
1	Benton Valley*	31 (4)	25		1	30
2	Chalfant Valley*	23 (2)	16			23
3	Volcanic Tableland	18 (2)	16			18
4	East of Laws	14 (3)	13		2	12
5	West of Laws	13 (4)	13		2	11
6	Tungsten Hills	15 (4)	15		9	6
7	Horton Creek	16 (3)	16		15	1
8	Southwest of Bishop	16 (4)	15		13	3
9	South of Bishop	18 (6)	17	2	12	4
10	Southeast of Bishop	13 (2)	13		11	2
11	West of Black Canyon	13 (4)	13		9	4
12	Big Pine	15 (2)	14		14	1
13	Independence	20 (2)	20		19	1
14	Coso Junction*	18 (6)	8	2	14	2
Ref	Jawbone Canyon*	15 (3)	11	1	14	
Totals		273 (53)	240 (238)	5	135	133

[a] Asterisks indicate samples analyzed previously by Johnson and Marten (1992). Two sites (Rattlesnake Flat and Jawbone Canyon) were included as references away from the Owens Valley area of potential contact. Haplotype group designation follows C. Cicero and N. K. Johnson (unpubl. data). Group AC, which is rare in *A. b. canescens* from Owens Valley and the Mojave Desert, is more common in populations of that taxon from the San Joaquin Valley of California.

[b] Number of individuals analyzed with PCR–RFLP (number of individuals sequenced in parentheses).

[c] Total number in parentheses represents specimens that were not missing morphometric data.

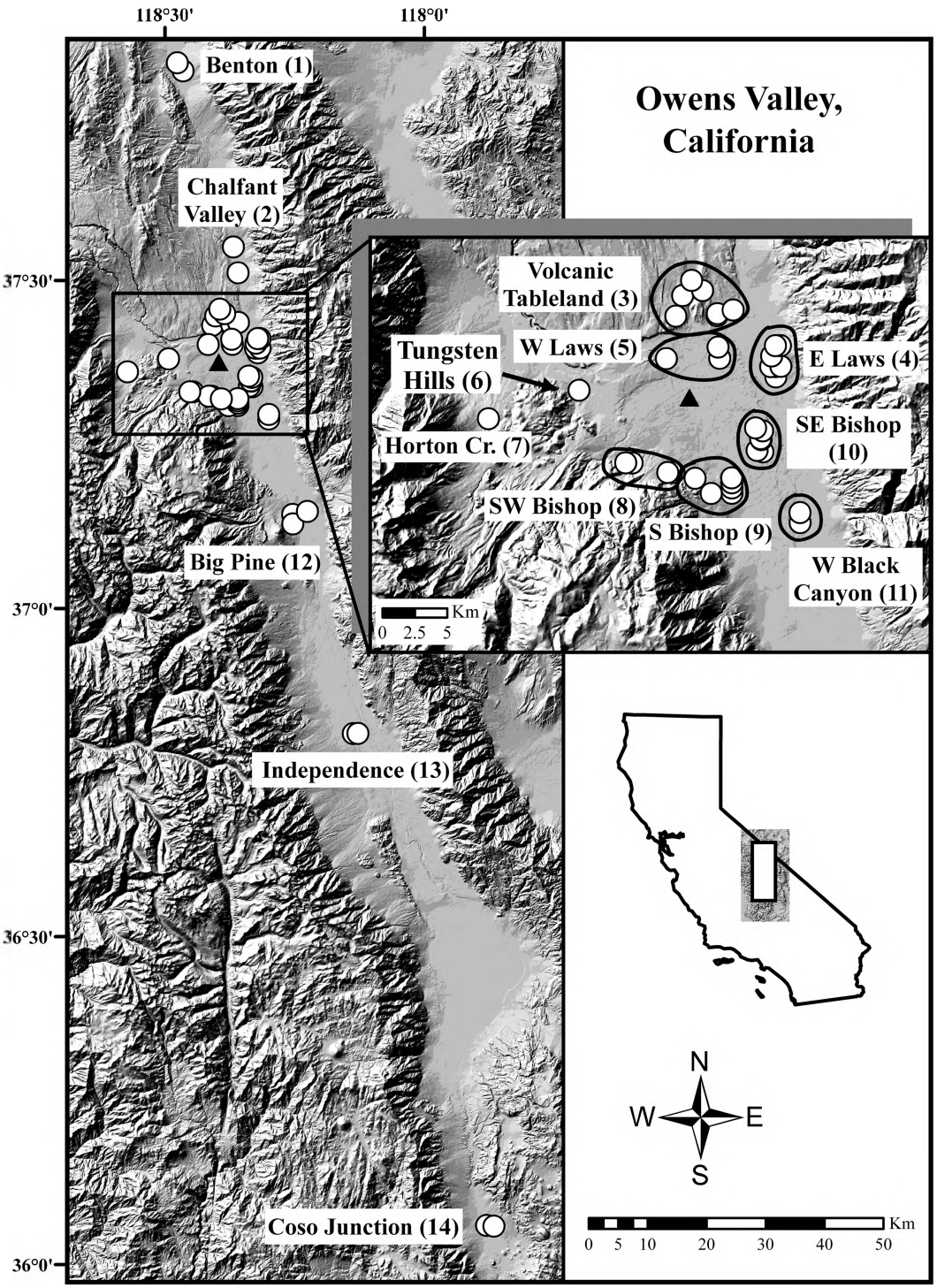

FIG. 2. Sampling locations for specimens of *Amphispiza belli* from the Owens Valley transect. Localities were grouped into 14 sample areas for analysis (see Table 1). Black triangle indicates location of Bishop, Inyo County, California. Bottom scale (inset) refers to the relief map of the entire valley.

Johnson and Marten 1992; see Fig. 1) revealed three primary haplotype groups, two of which are dominant in *A. b. nevadensis* and *A. b. canescens*, respectively, as they approach Owens Valley. These data were based on a combination of DNA sequencing and PCR–RFLP, a useful technique for classifying large numbers of individuals with respect to haplotype in phylogeographic or hybrid-zone studies (e.g., Rohwer et al. 2001, Ruegg and Smith 2002, Cicero 2004). We used the same approach to identify individuals of *A. belli* collected at each of the sample areas from Rattlesnake Flat to Jawbone Canyon (n = 273; Table 1).

Whole genomic DNA was extracted from fresh frozen tissue using a DNeasy tissue kit (Qiagen, Valencia, California), and a ~900 base-pair (bp) fragment of cytochrome *b* was amplified using primers L14987 and H15916 (Cicero and Johnson 2001; Table 2). A negative control was included in all extraction and amplification experiments. Two diagnostic restriction enzymes (Alu I, Hinf I) were used to digest the PCR products using the same protocol described by Cicero (2004), and fragments were visualized on 2.5% Nusieve:0.5% Seakem agarose gels stained with ethidium bromide. Individuals then were scored according to haplotype pattern. To confirm haplotype designations, and for population genetic analyses, a subset of 53 individuals (Table 1) was amplified and sequenced for the entire cytochrome-*b* gene (primers L14851–H15304, L15236–H15916, L15557–H16065 in Cicero and Johnson 2001; Table 2). Sequences were obtained with an ABI Prism 377 automated sequencer (Applied Biosystems, Foster City, California).

Sequences were analyzed for several population genetic measures using ARLEQUIN, version 3.01 (Excoffier et al. 2005) and DNASP, version 4.10 (Rozas et al. 2003). Analyses included (1) nucleotide diversity and its variance (Nei 1987), which estimates the mean number of nucleotide differences among haplotypes; (2) hierarchical analysis of molecular variance (AMOVA), which estimates genetic diversity among versus within subspecies; (3) Fu's F_S statistic (Fu 1997), which—assuming neutrality—can be used to infer demographic population expansion; (4) mismatch distributions (Slatkin and Hudson 1991, Rogers and Harpending 1992), which compare the frequency of observed versus expected pairwise differences between haplotypes to see whether they show patterns of an expanding population; and (5) pairwise M, which uses the island model of population structure (Wright 1951) to estimate gene flow (Nm) by the relationship $M = (1 - F_{ST})/2\,F_{ST}$.

Morphometrics.—Seven linear external measurements (mm) were taken on museum skins according to standard protocols (Johnson 1980, Cicero 1996) with dial calipers: wing length; tail length; bill length, depth, and width; tarsus length; and middle toe length without claw. In addition, body mass was recorded in the field and transformed to cube root for analysis. Morphological data were recorded from the same specimens used in molecular analyses to assess individual concordance between phenotype and genotype. Because our field sampling emphasized singing birds to obtain tape-recorded songs, most of the specimens were males; thus, morphological data were limited to adult males (n = 240; Table 1), whereas the molecular data set (n = 273) also included adult females. In addition to singing, all males were in breeding condition on the basis of enlarged gonads.

TABLE 2. Population genetic measures for two subspecies of *Amphispiza belli*, subdivided by geographic region.[a] Values are based on complete sequences (1,143 bp) of the cytochrome-*b* gene. Percentage of variation among subspecies within regions is indicated in parentheses (AMOVA).

Group	Sample size	Number of unique haplotypes	Nucleotide diversity	Fu's F_S [b]
Owens Valley (82.5–95.3%)				
nevadensis	15	4	0.0004 ± 0.0004	–2.369*
canescens [c]	21	9	0.0042 ± 0.0024	–0.074
	18	6	0.0011 ± 0.0008	–1.908
Great Basin and Mojave Desert–San Joaquin Valley (57.5%)				
nevadensis	15	4	0.0004 ± 0.0004	–2.369*
canescens	16	7	0.0078 ± 0.0043	2.963

[a] Analysis is based on sequenced specimens identified either as "pure" *A. b. nevadensis* or "pure" *A. b. canescens* on the basis of both diagnostic mtDNA haplotypes and discriminant-function scores (*nevadensis*, haplotype group BC, score > 0; *canescens*, haplotype groups AD or AC, score < 0; see Figs. 3 and 4). Thus, possible hybrid or intermediate individuals within Owens Valley (i.e., those with a haplotype diagnostic of one subspecies but morphologically classified as the other subspecies) were excluded from analysis.

[b] Values significant at $P \leq 0.05$ are indicated by an asterisk. Fu's F_S for *A. b. canescens* from Owens Valley (–1.908) is barely nonsignificant (P = 0.07).

[c] Upper values for *A. b. canescens* include all "pure" individuals (see footnote a) in haplotype groups AD or AC (AMOVA among-subspecies variation = 82.5%). Lower values exclude three birds in the haplotype group AC (two at Coso Junction, one at Jawbone Canyon; Table 1), which characterizes *A. b. belli* and San Joaquin populations of *A. b. canescens* (C. Cicero and N. K. Johnson unpubl. data) but is rare in the Owens Valley region (AMOVA among-subspecies variation = 95.3%).

[d] Patterns outside the Owens Valley region are shown for comparison. The lower among-subspecies variation (AMOVA = 57.5%) is attributable to the presence of two distinct haplotype groups within *A. b. canescens* (C. Cicero and N. K. Johnson unpubl. data). Rattlesnake Flat, Benton Valley, Chalfant Valley, Coso Junction, and Jawbone Canyon were included in both analyses (see text).

Definitive breeding status is an important criterion when including individuals for geographic studies (Cicero and Johnson 2006).

Morphological variation was studied by stepwise discriminant-function analysis (DFA) using STATISTICA, version 6.0 (Statsoft, Tulsa, Oklahoma). Specimens were grouped by geographic sample for analysis, using Rattlesnake Flat and Jawbone Canyon as references away from Owens Valley. In addition, specimens were classified according to haplotype group (AD, BC; Table 1) to assess morphological versus genetic patterns at the level of individual birds. The five individuals who shared a third rare haplotype in the region (AC; Table 1) were combined with haplotype AD, because both represent *A. b. canescens* on a broader geographic scale (C. Cicero and N. K. Johnson unpubl. data). Comparison of AC and AD individuals in this region did not reveal any significant differences in size.

Bioclimatic and landcover data.—Bioclimatic and landcover data were obtained from online sources to examine climatic and vegetational trends across the Owens Valley region (Benton Valley to Coso Junction). For the bioclimatic analysis, 11 temperature (°C) and 8 precipitation (mm) variables were downloaded from the WORLDCLIM data set (see Acknowledgments). Unique locations (n = 40) for the 14 core sample areas were georeferenced to obtain latitude and longitude coordinates in decimal degrees, and bioclimatic variables were extracted for points at each site using DIVA-GIS, version 5.2 (Hijmans et al. 2005). Variables were analyzed across sites with principal-component analysis (STATISTICA, version 6.0). In addition, ecological-niche models for *A. b. nevadensis* and *A. b. canescens* were generated, along with those for *A. b. belli,* by the maximum-entropy method (Maxent; Elith et al. 2006; Phillips et al. 2004, 2006), using BIOCLIM variables and range-wide georeferenced localities for breeding specimens (C. Cicero and N. K. Johnson unpubl. data); these were used to visualize predicted distributions of the two subspecies in the Owens Valley.

Landcover data, including 77 landcover classes in the wildlife habitat relationships (WHR) system and a hierarchical classification of WHR types into 10 "major landcover" classes (WHR10), were obtained from the California Department of Forestry Fire and Resource Assessment Program (see Acknowledgments). Major landcover classes were mapped onto the Owens Valley region to examine geographic concordance between habitat type, Maxent predictions, and molecular or morphological transitions. All maps were generated using ARCMAP, version 9.1 (ESRI, Redlands, California).

Results

*Sharp transition of cytochrome-*b *haplotypes in the northern Owens Valley.*—Of the 273 samples analyzed genetically using PCR–RFLP, including Rattlesnake Flat and Jawbone Canyon, 48.7% (n = 133) were classified in the BC haplotype group and 49.5% (n = 135) in the AD group (Table 1); the remaining five individuals (1.8%) had a haplotype characteristic of *A. belli* populations farther to the west (C. Cicero and N. K. Johnson unpubl. data). On a broader geographic scale, BC is typical of *A. b. nevadensis*, whereas AD is most common in *A. b. canescens* from the Mojave Desert. The distribution of haplotype groups across the 14 core sample areas in the Owens Valley region was not random. Thus, BC was dominant in samples from Benton Valley, Chalfant Valley, Volcanic Tableland, and near Laws, an old railroad station northeast of Bishop, Inyo County (Table 1 and Fig. 2); all these sites occur at the extreme northern end of the Owens Valley or just to the north. South of Bishop, the dominant haplotype group changed abruptly from BC to AD, a pattern that continued southward to Coso Junction at the extreme southern end of the Owens Valley region. Haplotype AD also dominated at Horton Creek, a tributary of the Owens River that flows north-northeast ~16 km west of Bishop. Interestingly, this haplotype appears to be rare in the more northern samples (only 5.1% of individuals from Benton Valley to Laws), whereas BC occurs in low to rare frequency all the way from Bishop to Coso Junction (15.3% of individuals). The most mixed sample occurs to the west of Bishop at Tungsten Hills, where both haplotypes were found in high frequency (AD = 60%; BC = 40%). Overall, estimated mtDNA gene flow between the two subspecies in this region was low (M = 0.024–0.106, depending on whether the rare AC haplotype group was included in the analysis); values greater than one are expected to prevent populations from differentiating solely because of drift (Slatkin 1993, Mills and Allendorf 1996).

Morphological patterns are congruent with genetic data.—Patterns of size variation detected by DFA generally were congruent with cytochrome-*b* variation in haplotype groups. Plots of discriminant-function scores for the two reference samples away from Owens Valley (*A. b. nevadensis* at Rattlesnake Flat and *A. b. canescens* at Jawbone Canyon; Fig. 3) showed significantly different distributions (*A. b. nevadensis* [score > 0] larger than *A. b. canescens* [score < 0]; Kolmogorov-Smirnov two-sample test, $P < 0.001$), which matched results for the

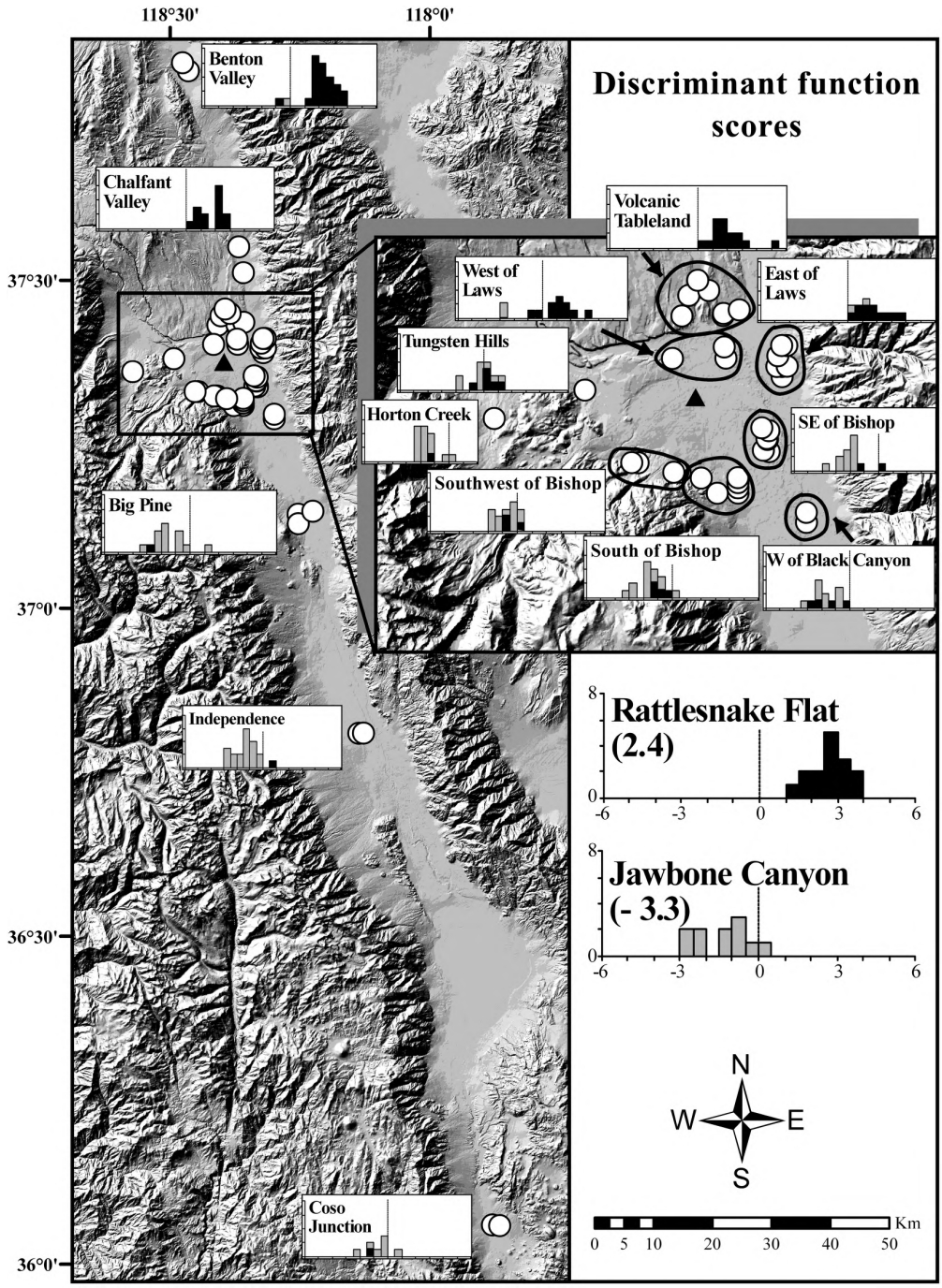

FIG. 3. Discriminant-function analysis of size characters for male *Amphispiza belli* (n = 212) in the Owens Valley region, grouped by sample location (Table 1) and categorized by mtDNA haplotype group (black bars = *A. b nevadensis* [BC]; gray bars = *A. b. canescens* [AD and AC]). Discriminant-function scores of reference males from Rattlesnake Flat and Jawbone Canyon, outside the study area (see Fig. 1), were significantly different (Kolmogorov-Smirnov two-sample test, $P < 0.001$; mean scores in parentheses). Vertical bars in discriminant-function plots show a score of zero.

two subspecies across their geographic range (Cicero and Johnson 2006). Within the Owens Valley region, discriminant-function scores showed a clear trend from larger birds in the north (score > 0; e.g., Benton Valley, Chalfant Valley, Volcanic Tableland, East and West of Laws) to smaller birds in the south (score < 0; e.g., Horton Creek and Bishop south to Coso Junction). As with mtDNA, the most morphologically intermediate sample occurred at Tungsten Hills.

Analysis of mtDNA by morphology (Fig. 3) showed that most of the birds with a discriminant-function score > 0 have a haplotype in the BC group (*nevadensis*), whereas those with scores < 0 have either an AD or AC haplotype (*canescens*). Although samples from near Bishop (southwest of Bishop, south of Bishop, and west of Black Canyon) were more mixed, with relatively small birds (size typical of *A. b. canescens*) having BC haplotypes characteristic of *A. b. nevadensis*, 85.4% of individuals from the 14 core sample areas were correctly classified according to mtDNA by size. Thus, of the 212 males in the analysis, 80% of birds with a BC haplotype (n = 99) were classified as BC according to size, and 90% of birds with an AD or AC haplotype (n = 113) were classified by size in that group (Fig. 4). Although one would expect some recombinants to be like pure parentals, even in a hybrid swarm, it is noteworthy that most of the discriminant-function misclassifications occurred in the narrow zone where the two haplotypes meet near Bishop.

Despite the high proportion of correctly classified individuals in the region, application of a diagnosability index D_{ij} for subspecies (Patten and Unitt 2002) resulted in nondiagnosable differences in wing length between *A. b. nevadensis* and *A. b. canescens* in Owens Valley (D_{nc} = –1.00, D_{cn} = –1.23). In this analysis, individuals were assigned to one of the two subspecies on the basis of their discriminant-function score (>0 or <0, respectively) and haplotype group (BC vs. AD/AC). Thus, potentially intermediate individuals (i.e., those with a size typical of one subspecies and haplotype characteristic of the other) were excluded. Although this result contrasts with the discriminant-function data and with the strong diagnosability between *A. b. nevadensis* and *A. b. canescens* on a broader geographic scale (D_{nc} = 0.40, D_{cn} = 1.01; Cicero and Johnson 2006), D_{ij} relies on a single morphological trait. Furthermore, reduced diagnosability between subspecies is expected in areas of contact because of intergradation, selection, or both, in a common environment.

Population genetic measures suggest rapid expansion into Owens Valley.—Sequencing of haplotypes for a subset of individuals from Owens Valley revealed four unique types in *A. b. nevadensis* and nine in *A. b. canescens* (Table 2), with an average nucleotide difference of 1.4% (six haplotypes in *canescens* and 1.6% difference if individuals with the rare AC group are excluded). According to the AMOVA, nearly all the molecular variance (82.5–95.3%) is apportioned between rather than within subspecies in that region—in contrast to the pattern outside of Owens Valley, where the high variance within subspecies is attributable to the division of *A. b. canescens* into two haplotype groups (AD and AC) separated geographically (Mojave Desert vs. San Joaquin Valley, respectively; C. Cicero and N. K. Johnson unpubl. data). The higher haplotype diversity found in *A. b. canescens* is consistent with its observed nucleotide diversity, which is approximately an order of magnitude greater than that for *A. b. nevadensis* in both the Owens Valley and broader regions (Table 2).

Both Fu's (1997) F_S statistics and mismatch distributions suggest rapid demographic expansion into Owens Valley, though the patterns are also consistent with a recent selective sweep in *A. b. nevadensis*. Fu's F_S (Table 2) is negative for *A. b. nevadensis* (–2.369) and *A. b. canescens* (AD haplotype group, –1.908) in this region, indicating an excess of new mutations in relation to equilibrium expectations on the basis of the number of observed alleles. Similar results were obtained more broadly for *A. b. nevadensis* in the Great Basin (F_S = –2.369). By contrast, an opposite positive pattern (F_S = 2.963) was observed in populations of *A. b. canescens* from the Mojave Desert–San Joaquin Valley. The unimodal mismatch distributions (Fig. 5) for the two subspecies in the Owens Valley region did not differ significantly from that expected under a sudden-expansion model.

Owens Valley transect occurs along ecological and bioclimatic gradients.—Principal-component analysis of the 19 bioclimatic variables showed a strong latitudinal gradient among sites in the Owens Valley region (Fig. 6A)—from Coso Junction in the south (hot and dry Mojave

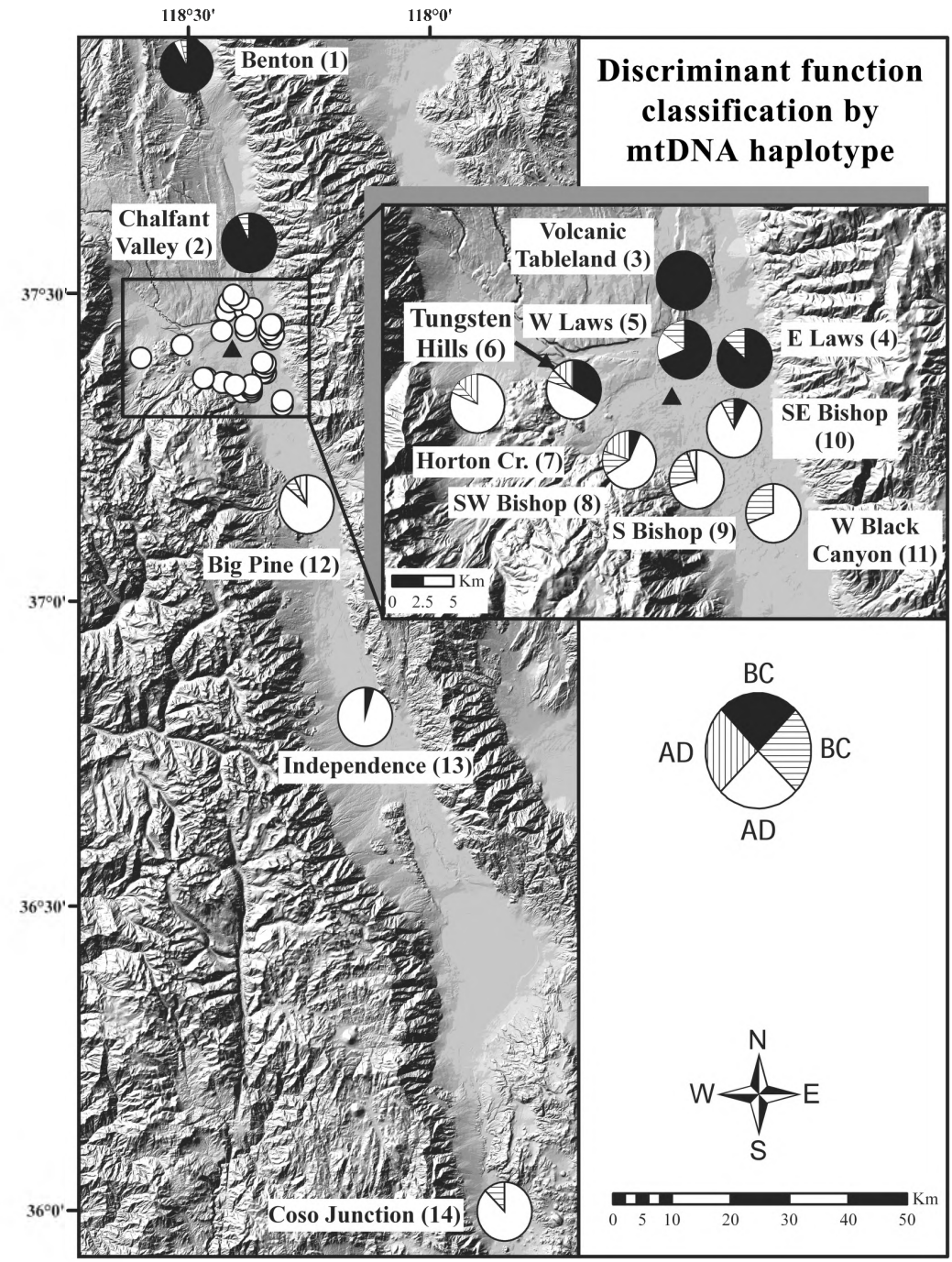

FIG. 4. Discriminant-function classification of male *Amphispiza belli* (n = 212) in the Owens Valley region, grouped by sample location (Table 1) and categorized by mtDNA haplotype. Pie diagrams show the percentage of specimens with *A. b. nevadensis* (BC) or *A. b. canescens* (AD or AC) haplotypes classified correctly (solid black or white, respectively) versus incorrectly (hatched patterns)—that is, *nevadensis* haplotype classified by size as *nevadensis*, *canescens* haplotype classified by size as *canescens*. Right hatching = *canescens* haplotype classified by size as *nevadensis*. Left hatching = *nevadensis* haplotype classified by size as *canescens*.

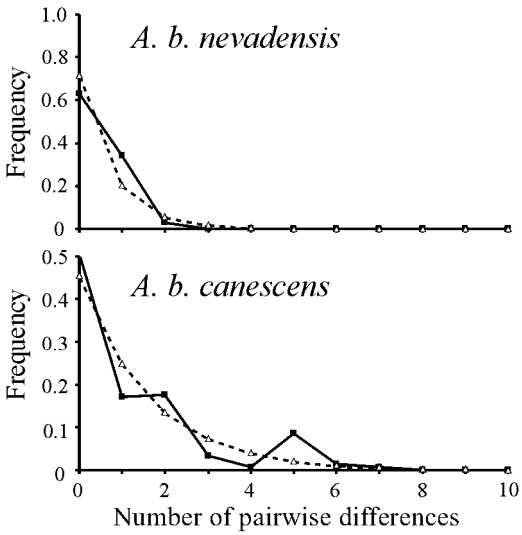

FIG. 5. Mismatch distributions for *A. b. nevadensis* and *A. b. canescens* in the Owens Valley region, on the basis of complete sequences of cytochrome *b* (closed square symbols–solid line = observed; open diamond symbols–dashed line = expected). Analysis of *A. b. canescens* excluded three individuals with sequences of the AC haplotype group, which is diagnostic for populations in the San Joaquin Valley and is rare in Owens Valley and the Mojave Desert. Observed and expected distributions are not significantly different ($P > 0.05$) and are consistent with rapid range expansions into the region.

Desert) to Benton Valley in the north (cold and wet Great Basin Desert). Localities in the contact zone clustered together toward the colder and wetter end of the gradient. In the analysis, the first two principal-component axes contained 88.4% of the variation and reflect overall temperature and precipitation conditions (PC1) versus winter and seasonal variation (PC2), respectively (Table 3). Benton Valley and Chalfant Valley (*A. b. nevadensis*) were most similar along PC1 (Fig. 6B), whereas Coso Junction and Independence (*A. b. canescens*) clustered near each other on PC2. The two sites closest to the contact zone (Chalfant Valley and Big Pine) were ecologically similar to those within the zone near Bishop. Interestingly, Horton Creek at the west end of the contact zone is an outlier with regard to climate, with PC1 being most similar to Benton Valley and PC2 reflecting conditions similar to those in Coso Junction. Individuals at that site are primarily *A. b. canescens* according to haplotype and morphology (Figs. 3 and 4), but occupy habitat of sagebrush and bitterbrush (*Purshia tridentata*) similar to that of *A. b. nevadensis*.

Ecological-niche models of *A. b. nevadensis* and *A. b. canescens* across their geographic ranges (C. Cicero and N. K. Johnson unpubl. data) provide more insight into the bioclimatic transition across Owens Valley (Fig. 7). Sites with high suitability for *A. b. nevadensis* range from Benton Valley south through Bishop and the contact zone, with Big Pine occurring at the southern end of suitable bioclimate; Independence and Coso Junction are unsuitable. On the other hand, sites with highest suitability for *A. b. canescens* include Coso Junction and Independence. According to these models, Horton Creek is unsuitable for either form. Importantly, there is essentially no overlap in Owens Valley between predicted models on the basis of bioclimatic variables.

Whereas the bioclimatic data point to a transition south of Bishop, with contact-zone sites situated in areas more suitable for *A. b. nevadensis*, the major landcover classes (WHR10) show a slightly different picture (Fig. 7). According to those layers, the vegetation makes a transition from "desert" (e.g., alkali desert scrub) to "shrub" (e.g., sagebrush) between Benton and Chalfant valleys, which is consistent with our personal field experience. Thus, sites from Chalfant Valley south to Coso Junction occur mostly in scrubby habitat more characteristic of *A. b. canescens* than *A. b. nevadensis*. However, those on the west slopes of Owens Valley (just above the valley floor) occur in an area of interdigitization between "desert" and "shrub" classes. The most notable exception again is Horton Creek, which falls purely in "shrub" comparable to habitat in Benton Valley.

DISCUSSION

Studies of contact zones provide critical insight into evolutionary processes and barriers to sympatry between differentiated taxa (Cicero 2004, Swenson 2006). Because such zones often occur in environmental ecotones, an important goal of these studies should be to understand population-level patterns of variation in the context of climatic and ecological gradients. By combining molecular, morphological, and environmental data to examine areas of contact, distributional limits and evolutionary barriers to gene exchange between taxa become readily

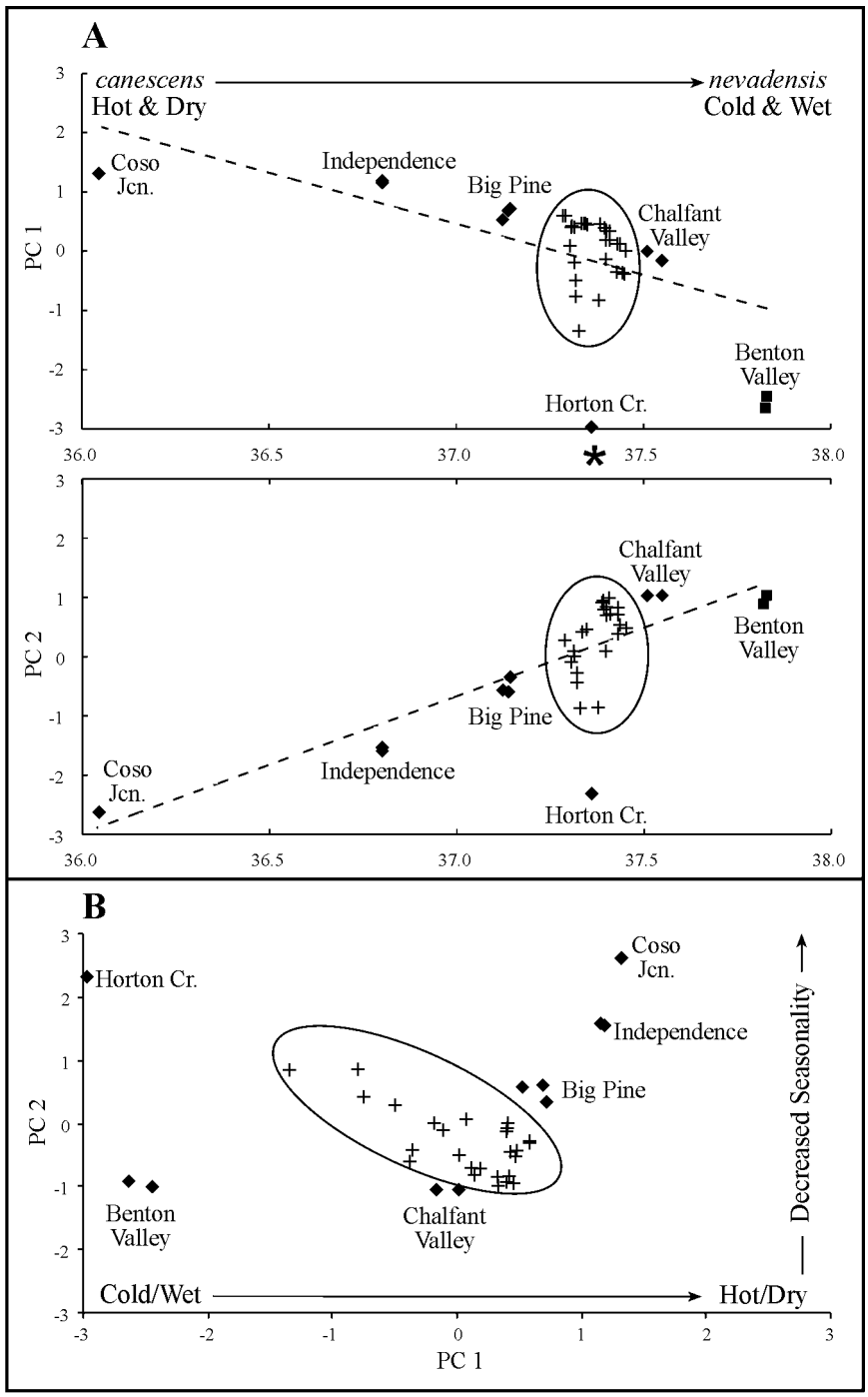

FIG. 6. Principal component scores for sample localities using 19 bioclimatic variables (Table 3) for analysis. (A) Sample localities plotted against latitude (*x*-axis) for PC1 and PC2. (B) Sample localities plotted for PC1 against PC2. In all graphs, sites along the transect are labeled with sample names (Table 1); those within the ellipsoid and shown by plus (+) symbols are in the area of contact between *A. b. canescens* and *A. b. nevadensis* near Bishop, Inyo County, California (latitude of Bishop indicated by asterisk).

TABLE 3. Factor loadings for 19 bioclimatic variables on the first three principal-component (PC) axes, based on analysis of sites along the Owens Valley transect. See text for definitions of variables.

	Bioclimatic variable	PC1	PC2	PC3
Bio1	Annual mean temperature	0.957	0.277	0.056
Bio2	Mean diurnal range	0.203	–0.874	–0.437
Bio3	Isothermality	–0.142	–0.723	–0.634
Bio4	Temperature seasonality	0.887	–0.148	0.356
Bio5	Maximum temperature of warmest month	0.986	–0.126	–0.081
Bio6	Minimum temperature of coldest month	0.716	0.674	0.128
Bio7	Temperature annual range	0.498	–0.824	–0.226
Bio8	Mean temperature of wettest quarter	0.845	0.473	0.089
Bio9	Mean temperature of driest quarter	0.900	0.098	–0.367
Bio10	Mean temperature of warmest quarter	0.969	0.220	0.102
Bio11	Mean temperature of coldest quarter	0.919	0.378	–0.048
Bio12	Annual precipitation	–0.783	0.579	–0.214
Bio13	Precipitation of wettest month	–0.615	0.722	–0.310
Bio14	Precipitation of driest month	–0.936	–0.235	–0.094
Bio15	Precipitation seasonality	0.297	0.871	–0.292
Bio16	Precipitation of wettest quarter	–0.636	0.724	–0.262
Bio17	Precipitation of driest quarter	–0.904	–0.215	0.357
Bio18	Precipitation of warmest quarter	–0.833	–0.107	0.533
Bio19	Precipitation of coldest quarter	–0.640	0.717	–0.269
Eigenvalue	11.086	5.724	1.741	
Total variance explained (%)		58.3	30.1	9.2

apparent (e.g., Dessauer et al. 2000, Cicero 2004). Furthermore, these data can be used to infer whether contact zones associated with environmental gradients are best explained as the most likely sites of sympatry between differently adapted parental taxa (Arnold 1997, Case and Taper 2000) or as a balance between dispersal and selection across the gradient (Barton and Hewitt 1985).

Owens Valley is an ideal setting for the study of avian contact zones for several reasons: (1) it is at the western end of the Great Basin, which is well known as an area of approach or contact in numerous avian taxa (Johnson 1978); (2) the valley is situated steeply between two sets of mountains—the Sierra Nevada to the west and the White-Inyo ranges to the east—and thus any areas of contact must be confined to a north–south geographic gradient; and (3) two major ecological regions (Great Basin and Mojave Desert) meet at the northern end of the valley. Results of this study show that *A. b. nevadensis* and *A. b. canescens* occur in narrow secondary contact where these ecological zones undergo transition. Furthermore, this contact appears to be fairly recent, as indicated by indirect evidence for rapid population expansion into Owens Valley and by paleobotanical evidence for lowland woodland habitat in both deserts during recent glacial cycles (Koehler and Anderson 1994, Cicero 1996 and references therein, Woolfenden 1996). Especially interesting is the finding that populations of *A. b. canescens* outside the valley show a signature of a stable or declining rather than expanding population (positive value for Fu's F_S), whereas *A. b. nevadensis* shows evidence of expansion throughout its range. Thus, the Mojave Desert may be acting as a source of individuals dispersing northward into Owens Valley toward its transition with the Great Basin.

The morphological and molecular data, as well as the sharpness (~10 km) and location of the contact zone, suggest that mtDNA introgression between *A. b. nevadensis* and *A. b. canescens* is concentrated toward the northern end of Owens Valley, near the breeding limits of the two subspecies. Furthermore, *nevadensis* mtDNA haplotypes appear to be more common in *canescens*-sized birds than vice versa (i.e., *canescens* haplotypes in *nevadensis*-sized birds) in this area. This pattern may be explained by directional interbreeding of *nevadensis* females with *canescens* males, selection for smaller size in *nevadensis* as an adaptation to hotter and drier conditions, or both. Difference in timing of breeding is also an important consideration. Reproductive data show that *A. b. canescens*

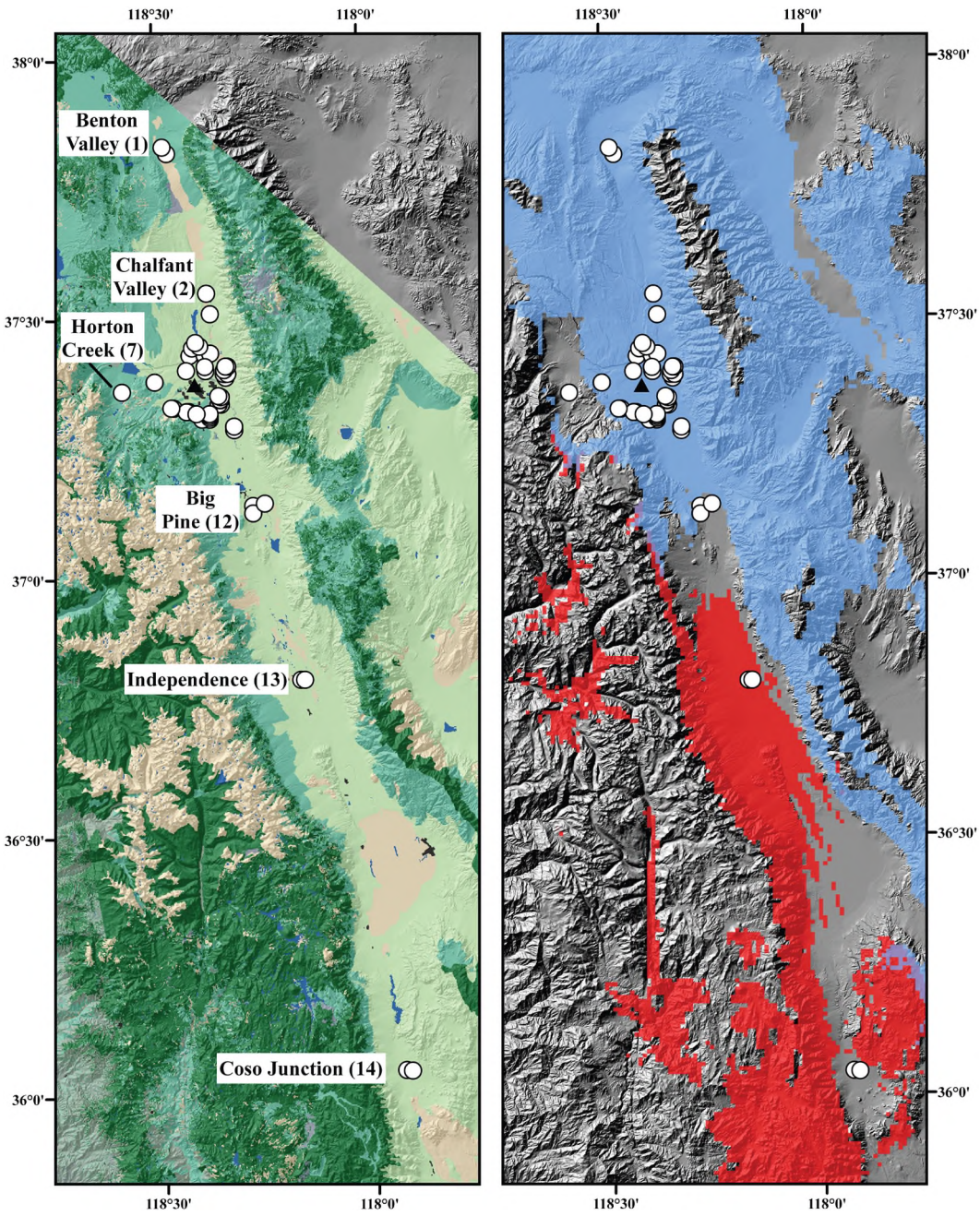

FIG. 7. (Left) Distribution of major landcover classes of wildlife habitat relationship (WHR10) for the Owens Valley transect (see text). Lightest green = "desert" (e.g., alkali desert shrub), darker green = "shrub" (e.g., sagebrush), and darkest green = conifer (not suitable for Sage Sparrows); tan = barren or agriculture. This layer is available only for California. (Right) Ecological niche models predicted for *A. b. nevadensis* (blue) and *A. b. canescens* (red) within the Owens Valley transect. Predictions are based on an analysis of bioclimatic variables and specimen points across the geographic ranges of each taxon, respectively (see text; C. Cicero and N. K. Johnson unpubl. data). Open circles in both panels show sample locations for the study (Table 1 and Fig. 2). Black triangle indicates the location of Bishop, California.

breeds earlier than *A. b. nevadensis* and that the latter subspecies often migrates northward through the range of actively breeding *canescens* (Cicero and Johnson 2006). Although this provides opportunities for interbreeding between *nevadensis* and *canescens*, such occurrences seem rare until *nevadensis* approaches the northern valley, where a steep gradient in climate and habitat favors *A. b. nevadensis* to the north and *A. b. canescens* to the south. Phenological differences may also explain the pattern at Horton Creek, where seasonal conditions typical of the breeding range of *canescens* (PC2 bioclimatic score similar to Coso Junction) likely favor earlier arrival by that subspecies, which would then start defending territories before the spring arrival of *nevadensis*.

The narrow contact zone between *A. b. nevadensis* and *A. b. canescens* at the northern end of Owens Valley is consistent with separate histories of isolation, adaptation, and genetic drift in distinctive vegetation–climate regions (e.g., see Cicero 2004). However, ecological and climatic transitions are not completely concordant in this region, with changes in landcover (sagebrush to desert scrub) apparent in Benton Valley, Mono County, whereas predicted distributional limits of *nevadensis* and *canescens* using bioclimate occur just south of Bishop, Inyo County. This difference in habitat near Benton Valley may be the reason that Grinnell and Miller (1944) surmised intergradation in that region. The observation that *nevadensis* occupies habitat more typical of *canescens* in the vicinity of Bishop, and that *canescens* occurs in habitat characteristic of *nevadensis* at Horton Creek, suggests that climate rather than vegetation type *per se* may be more important in influencing breeding occurrences. On the other hand, northernmost populations of *canescens* near Bishop experience bioclimates more similar to those of *nevadensis* in the Great Basin than to those of other populations in the Mojave Desert. This tension between suitable climate and habitat, and the ability of both forms to adapt locally to such marginal conditions, likely limits the geographic extent of contact and overlap (e.g., see Cicero 2004, Swenson 2006).

Whether *A. b. nevadensis* and *A. b. canescens* breed sympatrically can be inferred by examining the taxonomic assignment of individual birds on the basis of both mtDNA and morphology. Away from the contact zone, evidence for limited sympatry is found at Benton Valley (one breeding *A. b. canescens*, MVZ 180128) and Independence (one breeding *A. b. nevadensis*, 173336). In addition, exploratory sampling east of Owens Valley at Palmetto Wash, Esmeralda County, Nevada, revealed one *nevadensis* (MVZ 173787) in a breeding population of mostly *canescens* (MVZ 165425–165427, 173767, 173788–173790, 177065–177066; n = 9). Sympatry within the contact zone is most evident west of Laws, where two unequivocal *canescens* (MVZ 177045, 178084) were collected in a sample otherwise dominated by *nevadensis* (see Fig. 3). If free interbreeding were occurring, one would expect more admixture of individuals (misclassification by morphology and mtDNA haplotype) across the region of eastern California and western Nevada where *nevadensis* and *canescens* potentially come to contact. Instead, the data clearly show diagnosable forms that are connected by sharp clines in morphology and mtDNA, and these clines are strongly correlated with climate and habitat where the Great Basin and Mojave Desert meet narrowly in the northern Owens Valley. The analysis of vocal differences between these same individuals (C. Cicero and N. K. Johnson unpubl. data) will provide additional data on their diagnosability in this region.

Although *A. b. nevadensis* and *A. b. canescens* do not appear to be freely interbreeding, the close tracking of morphology and mtDNA with ecology suggests that they may be experiencing extrinsic selection that could maintain narrow clines even in the face of extensive hybridization. The misclassification of a proportion of birds (~15%) across the zone suggests either some hybridization or size differences maintained by selection, or both. Because patterns of morphological variation can be equivocal in regard to hybridization across contact zones (Sattler and Braun 2000), determining the extent of interbreeding will require additional study of either nuclear genetic data or mated pairs, or both. Collection of mated pairs is difficult, because females are often sitting on nests and difficult to observe during the core breeding season, whereas males are singing and more visible. Nuclear genetic data can provide indirect evidence for underlying levels of gene flow, but such analysis requires a large number of loci, and especially nondiagnostic ones that are not under selection (Sattler

and Braun 2000, Brumfield et al. 2001). Further study of *A. belli* will incorporate vocal and nuclear genetic data to unequivocally address the question of whether *A. b. nevadensis* and *A. b. canescens* should be regarded as biological species, *contra* previous treatments that have wrongly lumped the two forms on the basis of phenotype (Rising 1996, Patten and Unitt 2002; but see Cicero and Johnson 2006). Likewise, ongoing study (C. Cicero and N. K. Johnson unpubl. data) is addressing the issue of relationships between these subspecies and coastal populations (especially *A. b. belli*).

Acknowledgments

The California Department of Fish and Game, U.S. Fish and Wildlife Service, and University of California Berkeley Animal Care and Use Committee issued the necessary permits and protocols for collection of specimens. C. Marchis performed the DNA laboratory work. R. Hijmans, M. Koo, W. Monahan, J. Fang, and K. Yamamoto assisted with the georeferencing and coordinate validation, GIS analyses, and preparation of maps. M. Braun, M. Matocq, and J. V. Remsen, Jr., provided useful comments on an earlier draft of this manuscript. We are grateful to all of these individuals for their assistance. All specimens, tissues, and recordings in this study are deposited in the collections of the Museum of Vertebrate Zoology, University of California, Berkeley (mvz.berkeley.edu). The WORLDCLIM data set is available at www.worldclim.org/bioclim.htm. Landcover data from the California Department of Forestry Fire and Resource Assessment Program is available at frap.cdf.ca.gov.

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APPENDIX. Specimens and GenBank numbers of *Amphispiza belli* examined using PCR–RFLP and sequencing of cytochrome *b*. A subset of specimens (adult males only) were analyzed morphometrically. Specimen details are available from the collections database of the Museum of Vertebrate Zoology (see Acknowledgments).

Sample name and number [a]		MVZ specimen number	GenBank accession numbers [b]
Ref	Rattlesnake Flat	166948–166952, 168571–168580	EF488686, EF488687
1	Benton Valley	168557–168570, 180117–180133	EF488688, EF488689, EF488690 , EF488691
2	Chalfant Valley	166960–166968, 169396–169398, 178279, 181710–181719	EF488692, EF488693
3	Volcanic Tableland	173310–173316, 176477, 177040–177044, 177046–177050	EF488694, EF488695
4	East of Laws	177155–177159, 177466–177470, 178080–178081, 178086–178087	EF488696, EF488697, EF488698
5	West of Laws	173317, 176478–176482, 177045, 177160, 177465, 178082–178085	EF488699, EF488700, EF488701, EF488702
6	Tungsten Hills	178065–178079	EF488703, EF488704, EF488705, EF488706
7	Horton Creek	178049–178064	EF488707, EF488708, EF488709
8	Southwest of Bishop	173321–173323, 173548–173552, 177057–177064	EF488710, EF488711, EF488712, EF488713
9	South of Bishop	173553–173558, 176496–176501, 177051–177056	EF488714, EF488715, EF488716, EF488717, EF488718, EF488719
10	Southeast of Bishop	173318–173320, 173761–173765, 176483–176487	EF488720, EF488721
11	West of Black Canyon	173324–173328, 176488–176495	EF488722, EF488723, EF488724, EF488725
12	Big Pine	173329–173333, 173766–173775	EF488726, EF488727
13	Independence	173334–173343, 173776–173785	EF488728, EF488729
14	Coso Junction	170321–170338	EF488730, EF488731, EF488732, EF488733, EF488734, EF488735
Ref	Jawbone Canyon	169351–169354, 170283–170293	EF488736, EF488737, EF488738

[a] Sample names and numbers refer to Table 1 and Figure 1. Rattlesnake Flat and Jawbone Canyon are reference samples outside the Owens Valley region.

[b] GenBank numbers are available only for sequenced individuals (n = 53). MVZ = Museum of Vertebrate Zoology, University of California, Berkeley.