MACROGEOGRAPHIC PATTERNS OF MORPHOMETRIC AND GENETIC VARIATION IN THE SAGE SPARROW COMPLEX[1]

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Abstract. We examined morphometric and genetic variation in 22 populations of three subspecies of the Sage Sparrow (*Amphispiza belli belli, A. b. canescens,* and *A. b. nevadensis*). The sum of squares simultaneous test procedure (SS-STP) demonstrated clear patterns of geographic change in six linear characters and in cube root of body mass. A UPGMA dendrogram and ordination plots using principal component analysis (PCA) and multidimensional scaling (MDS) defined two major groups of populations based on morphometric criteria: (I) those in the Coast Ranges and west slope of the Sierra Nevada (*A. b. belli*) plus those in the southern San Joaquin Valley and northern Mojave Desert (*A. b. canescens*), and (II) those in the Great Basin (*A. b. nevadensis*). Seventeen of 41 genetic loci scored (41.5%) were polymorphic. Genetic data identified the same two groups of populations delimited on morphometric grounds. Populations in group I were significantly less heterozygous than those of group II. Nei's genetic distances among populations of one taxon ranged from $\bar{D} = 0.0007$ (in *A. b. nevadensis*) to $\bar{D} = 0.0015$ (in *A. b. canescens*) and $\bar{D} = 0.0019$ (in *A. b. belli*). Intertaxon Nei's $\bar{D}$ ranged from 0.0027 (*A. b. belli* vs. *A. b. canescens*) to 0.0056 (*A. b. canescens* vs. *A. b. nevadensis*) and 0.0077 (*A. b. belli* vs. *A. b. nevadensis*). An overall mean F_{st} value of 0.112 points to pronounced genetic structuring of the 22 populations. Sedentary populations are less panmictic than migratory populations. Gene flow is estimated at 2–4 immigrants per generation. Although intergradation is unknown, *A. b. belli* and *A. b. canescens* are genetically closely related. In contrast, *A. b. nevadensis* and *A. b. canescens* differ strongly on both morphologic and genetic grounds.

Key words: Morphometrics; allozymes; geographic variation; gene flow; genetic and morphologic concordance; Sage Sparrow; Amphispiza belli.

INTRODUCTION

This is the second in a projected series of three papers on geographic differentiation in the Sage Sparrow (*Amphispiza belli*), an abundant passerine that occupies arid and semi-arid brushland in the western United States and Baja California, Mexico (Fig. 1). The first paper (Johnson and Cicero, in press) dealt with mitochondrial DNA (mtDNA) base sequence variation at the cytochrome b locus. The present paper offers a broad-scale analysis of morphometric and allozymic variation, the latter based on an electrophoretic survey of protein-coding loci, and will integrate information from these data sets in a discussion of population structure and gene flow. The final paper, based on work in progress, will present a microgeographic analysis of the interaction of two well-differentiated forms where they approach and probably contact in the vicinity of Owens Valley, California (N. K. Johnson, in prep.).

Because it is comprised of strongly characterized sets of populations that vary in size, plumage coloration, habitat selection, and migratory tendency, the Sage Sparrow complex is especially appropriate for a detailed examination of geographic variation. Birds from the foothills of the Coast Ranges and the western slope of the central Sierra Nevada in California (*A. b. belli*) are relatively small and heavily pigmented, inhabit chaparral in which chamise (*Adenostoma fascic-*
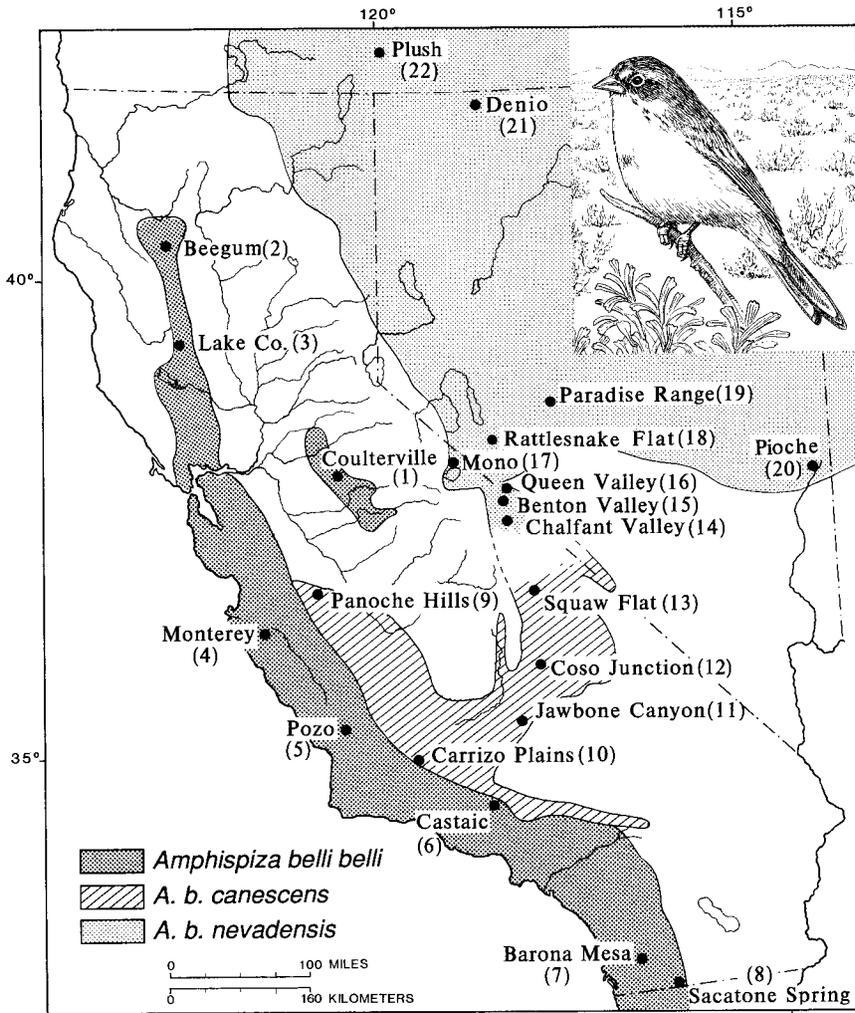
FIGURE 1.   The known nesting distribution of the Sage Sparrow in the far western United States. For California, the distribution is based mainly on Grinnell and Miller (1944:500), with exceptions noted in the text. The range of *A. b. belli* extends southward into northwestern Baja California and that of *A. b. nevadensis* extends northward to central interior Washington, eastward to southwestern Wyoming and northwestern Colorado, and eastward to northeastern Arizona and northwestern New Mexico (American Ornithologists' Union 1957, 1983). The distributions of two additional subspecies, the endangered *A. b. clementeae* of San Clemente Island and *A. b. cinerea* of central Baja California, are not shown.

ulatum) and/or California sagebrush (*Artemisia californica*) are dominant, and are essentially sedentary. Populations in the San Joaquin Valley and northern Mohave Desert (*A. b. canescens*), in contrast, are comprised of larger and much paler birds that prefer to nest in desert scrub in which saltbush (*Atriplex* sp.) is prevalent. Furthermore, they are migratory. Immediately following early spring breeding in the lowlands, these

populations undergo a peculiar and rapid uphill migration in late spring that takes them into the active nesting range of coastal and Sierran foothill birds. This phenomenon led Grinnell (1898) to suspect (incorrectly) that the two forms bred sympatrically. In late summer and fall, after a period of uncertain length spent in the highlands where they complete the major annual molt, these birds descend from the mountains and spread

southward and eastward to wintering grounds that include at least the southern portion of their breeding range in the southwestern United States. A third group of distinctive populations (*A. b. nevadensis*) breed in the interior of the western United States. These birds are large and pale, prefer brushland dominated by big sagebrush (*Artemisia tridentata*) or sagebrush-saltbush, and vacate most or all of their breeding range in an exodus to wintering regions in the southwestern United States and northern Mexico.

METHODS AND MATERIALS

We assumed that the Sage Sparrow was invariant in the geographic region under investigation, a null hypothesis that could be rejected only by the objective demonstration of significant geographic variation. Hence, subspecific names currently applied to populations in the United States were ignored when sites for sampling were chosen. However, once the fact of geographic variability was obvious, we elected to present our results in a framework of subspecies distributions in order to test their validity. As will become apparent, existing subspecific names admirably reflect the patterns of geographic variation that we identified.

COLLECTION OF SPECIMENS

This study was based upon 357 specimens taken mainly during the spring and early summer of 1977–1986 in California, Nevada, and southern Oregon. We divided this total into 22 samples for analysis (Fig. 1). Tissue for genetic study was available for all specimens but the morphometric analyses were confined to 274 males (range, 7–25 per sample; $\bar{x}$ = 12.5). Eight samples (Coulterville through Sacatone Spring) were from the coastal and Sierran foothill range ascribed to *A. b. belli*, five (Panoche Hills through Squaw Flat) were from the range of *A. b. canescens*, in the interior of south-central California, and the remaining nine (Chalfant Valley through Plush) were from the Great Basin portion of the distribution given for *A. b. nevadensis* (American Ornithologists' Union 1957). Because of complex post-breeding movement uphill of at least some birds that had bred in the San Joaquin Valley and northern Mohave Desert (*A. b. canescens*) into the range of *A. b. belli*, during the nesting period of the latter form, it was necessary to establish unambiguously that our analysis in-

cluded only breeding specimens. All samples except one (Squaw Flat) were of nesting individuals. The Squaw Flat sample, although comprised of non-breeders collected June 8–10(!), was taken to document early post-nesting migration to a single highland site by *A. b. canescens*. Specimens were mostly of singing males (except for the non-breeding, and hence non-singing, birds from Squaw Flat). Relatively few females were obtained because of their secretiveness during the breeding period. Finally, we attempted to take only one member of each pair of obviously mated birds in order to allow the remaining individual, usually the female, to raise the young.

MORPHOMETRICS

Body mass was measured to one-tenth gram (transformed to cube roots for analysis) with a Pesola balance within a few hours after collection. The majority of specimens were prepared as skin-skeletons (Johnson et al. 1984). The following measurements in millimeters were taken with a dial caliper from the dried preparations prior to skeletonization according to methods described in Johnson (1980:6–7): length of wing, length of tail, length of bill, depth of bill, width of bill, length of tarsus, and length of middle toe without claw.

Variation in single characters was first examined through the calculation of routine sample statistics. We then applied the sum of squares simultaneous test procedure (SS-STP; Gabriel 1964, Gabriel and Sokal 1969), using the program from Sokal and Rohlf (1969) as modified by Bruce Krogman into the program PAIRS. This procedure conducted an *a posteriori* analysis of variance on all samples for each character in order to identify maximally homogeneous (nonsignificant at $P \leq 0.05$) subsets of means.

After standardizing size characters by variance, we used NTSYS-pc (Rohlf 1988) to compute Euclidean distances among populations, to cluster populations with the unweighted pair group method (UPGMA), and to array them with principal components analysis (PCA) and multidimensional scaling (MDS, using MDSCALE). MDS was applied to the principal component plot and the original Euclidean distance matrix. Clustering resolves relationships at the level of fine branch tips, whereas ordination more accurately depicts larger groupings. Furthermore, MDS tends to preserve small intersample dis-

tances more faithfully than PCA (Rohlf 1988). A Minimum Spanning Tree was superimposed on the MDS plot to identify any distortion.

ALLOZYME ELECTROPHORESIS

We analyzed 41 protein-coding loci in tissue extracts using standard techniques described by Selander et al. (1971) and Yang and Patton (1981), with the slight modifications of Johnson et al. (1984). Protein assays were prepared according to Harris and Hopkinson (1976) and Selander et al. (1971). Electromorphs (alleles) at each locus were designated alphabetically in order of decreasing mobility. Seven loci (ACP, AK, EST-2, GLO, GR, ME, PHE-PRO) could not be scored consistently and were excluded. Two specimens of the Black-throated Sparrow from Nevada (*Amphispiza bilineata deserticola*) and three from Oklahoma (*A. b. opuntia*) were analyzed as a composite outgroup in the genetic comparisons. With 357 specimens from 22 populations examined at 41 loci (two alleles per locus = 29,274 total alleles), this study represents one of the most extensive genetic analyses of a wild species of bird.

Using BIOSYS-1 (Swofford and Selander 1981), we calculated observed and expected heterozygosities for each sample, χ^2 tests for departures from Hardy-Weinberg equilibrium, allelic frequencies, genetic distances (using the methods of Nei 1978 and Rogers 1972), and Wright's F_{st} (1965), using the modifications of Wright (1978) for small sample size and of Nei (1975) for multiple alleles. Because observed values of heterozygosity did not differ significantly from expected values based upon Hardy-Weinberg equilibrium (Nei 1975) in any comparison, only values for $H_{obs.}$ are presented. The relationship between morphologic distance and genetic distance was assessed with a Mantel test using the program MXCOMP in NTSYS-pc (Rohlf 1988).

Contemporary levels of gene flow were estimated with Wright's (1951) formula, $Nm = \frac{1}{4}\left(\frac{1}{F_{st}} - 1\right)$, and with Slatkin's (1981, 1985a, 1985b) rare allele method, which uses the formula $\ln p(1) = -0.505 \ln(Nm) + (-2.44)$, where $p(1)$ is the average frequency of private alleles and Nm is the product of the population size and immigration rate. Because our sample sizes differed among populations, we applied Slatkin's (1985a) recommended correction, in which Nm is divided by the ratio of the average sample size to 25. Thus, $Nm_c = Nm(\bar{N}/25)^{-1}$, where $\bar{N} = (\Sigma$ $N_i/n)$, the average sample size. N_i is the number of specimens in a given sample and n is the number of samples studied. For our average sample size of $\bar{N} = 16.2$, the correction factor was 0.649. Slatkin and Barton (1989) have verified the suitability of both F_{st} and rare alleles for estimating the average level of gene flow in natural populations as opposed to maximum likelihood methods.

MORPHOMETRIC RESULTS

UNIVARIATE ANALYSIS OF SIZE

Results of the SS-STP for wing length illustrate strong regional clumping of population means (Fig. 2, upper). Heading southward from the Inner Coast Range of northern California and on the west side of the Sierra Nevada, wing length gradually increases toward San Diego County (Barona Mesa, Sacatone Spring). These short-winged populations give way to birds of medium wing length across the arid interior of southern California. An abrupt shift occurs between Squaw Flat and Chalfant Valley, beyond which populations are long-winged across Nevada and into southern Oregon. Although the homogeneous subsets for tail length were more graded across all populations than were those for wing length, study of the map demonstrates definite geographic structure (Fig. 2, lower). Coastal birds were generally short-tailed; those from the southern California interior were medium in this feature, and Great Basin samples were comprised of relatively long-tailed birds. The strongest breaks were found between San Joaquin Valley (Panoche Hills, Carrizo Plains) and adjacent mid-coastal populations (Monterey, Pozo) and between samples near southern (Squaw Flat) and northern Owens Valley (Chalfant Valley).

Geographic variation in bill length was also clinal (Fig. 3, upper), but with a modest step between coastal and northern Mohave Desert populations and distinct shift in mean values between Squaw Flat and Chalfant Valley. Shortest-billed populations were coastal whereas all far interior populations had relatively long bills.

In contrast, bill depth (Fig. 3, lower) showed clinal variation along the coast, with population means lowest in San Diego County (Barona Mesa and Sacatone Spring) and highest in northern California (Lake Co.). San Joaquin Valley and northern Mohave Desert samples were intermediate. Again, Squaw Flat and Chalfant Valley contrasted sharply. Deep-billed populations be-

Coulterville (1)	64.6
Monterey (4)	65.7
Beegum (2)	65.9
Lake Co. (3)	66.3
Pozo (5)	66.4
Castaic (6)	67.6
Barona Mesa (7)	67.7
Sacatone Spring (8)	68.1
Carrizo Plains (10)	68.6
Panoche Hills (9)	68.7
Jawbone Canyon (11)	70.6
Squaw Flat (13)	71.2
Coso Junction (12)	71.2
Benton Valley (15)	76.1
Chalfant Valley (14)	76.4
Pioche (20)	76.7
Paradise Range (19)	76.8
Mono (17)	76.9
Queen Valley (16)	77.0
Rattlesnake Flat (18)	77.1
Denio (21)	77.1
Plush (22)	79.3
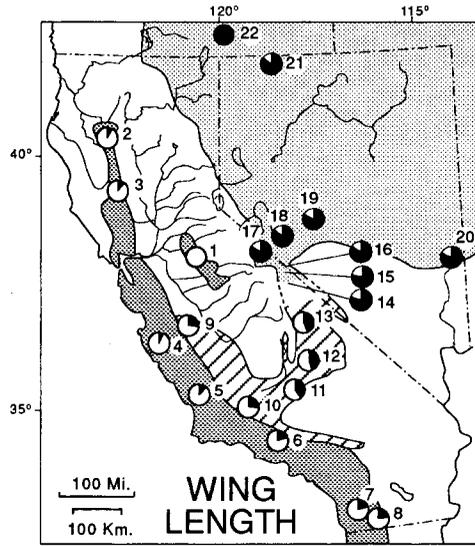
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Beegum (2)	65.6
Monterey (4)	65.7
Coulterville (1)	66.0
Pozo (5)	66.4
Lake Co. (3)	66.8
Barona Mesa (7)	67.0
Squaw Flat (13)	68.2
Castaic (6)	68.2
Sacatone Spring (8)	68.3
Carrizo Plains (10)	69.0
Coso Junction (12)	69.0
Jawbone Canyon (11)	69.0
Panoche Hills (9)	69.1
Pioche (20)	70.2
Benton Valley (15)	70.7
Denio (21)	70.8
Rattlesnake Flat (18)	71.4
Chalfant Valley (14)	71.4
Paradise Range (19)	71.5
Mono (17)	72.1
Queen Valley (16)	72.2
Plush (22)	73.6

WING LENGTH
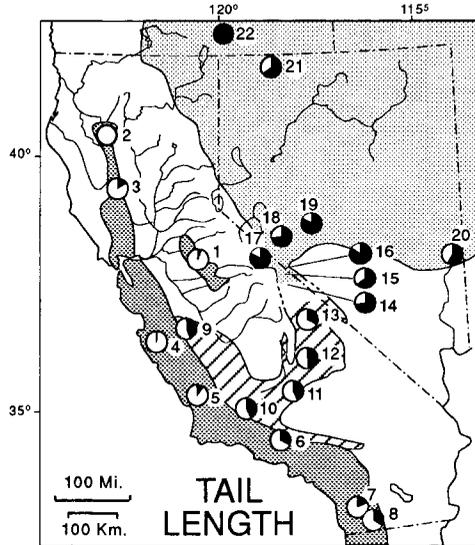
TAIL LENGTH

FIGURE 2. Sum of squares simultaneous test procedure (SS-STP) applied to population variation in wing length (upper) and tail length (lower) in *A. belli*. Populations are numbered as in Figure 1. The results are presented as maps of geographic variation with accompanying lists of means, ranked in order of magnitude, and placed next to the name and population number of each sample. Adjacent vertical lines denote the homogeneous subsets. For portrayal of the variation on each map, the total range of means for each character was converted to 100% and the pie diagrams for localities were scaled according to the relative magnitude of each mean. Because computation of homogeneous subsets was based on the values for the sample mean, sample size, and standard deviation, these diagrams obviate the need to present routine statistics of variation. The subsets are formed independent of geography; therefore, samples from widely disparate regions will be grouped together if their means are not significantly different.

gan at the latter site and continued across the Great Basin to eastern Nevada (Pioche), northern Nevada (Denio), and southern Oregon (Plush).

Change in bill width paralleled that seen in other features in that definite geographic clumping was evident (Fig. 4, upper). Populations in San Diego County (Barona Mesa, Sacatone Spring) and at Pozo had rather narrow bills. North coast, San Joaquin Valley, and northern Mohave

Beegum (2)	6.88
Pozo (5)	6.97
Barona Mesa (7)	7.02
Lake Co. (3)	7.06
Monterey (4)	7.08
Sacatone Spring (8)	7.11
Castaic (6)	7.20
Jawbone Canyon (11)	7.37
Coulterville (1)	7.39
Coso Junction (12)	7.48
Panoche Hills (9)	7.55
Squaw Flat (13)	7.61
Carrizo Plains (10)	7.68
Benton Valley (15)	7.96
Paradise Range (19)	8.02
Queen Valley (16)	8.04
Chalfant Valley (14)	8.10
Rattlesnake Flat (18)	8.12
Mono (17)	8.15
Pioche (20)	8.22
Denio (21)	8.29
Plush (22)	8.45
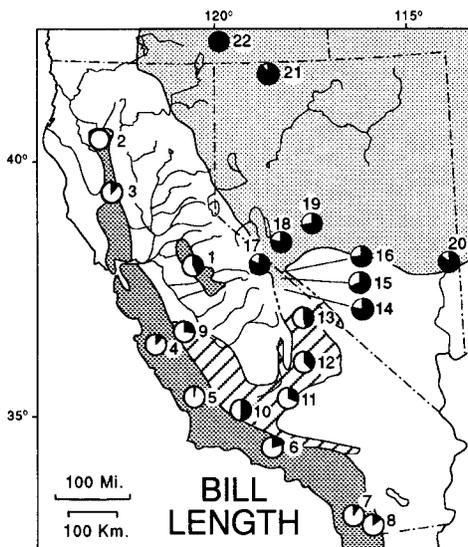
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Barona Mesa (7)	5.27
Sacatone Spring (8)	5.30
Jawbone Canyon (11)	5.35
Squaw Flat (13)	5.40
Coso Junction (12)	5.44
Carrizo Plains (10)	5.50
Panoche Hills (9)	5.53
Pozo (5)	5.53
Monterey (4)	5.58
Castaic (6)	5.59
Coulterville (1)	5.62
Paradise Range (19)	5.63
Queen Valley (16)	5.65
Beegum (2)	5.66
Chalfant Valley (14)	5.69
Benton Valley (15)	5.72
Rattlesnake Flat (18)	5.72
Mono (17)	5.75
Plush (22)	5.80
Lake Co. (3)	5.82
Denio (21)	5.83
Pioche (20)	5.91
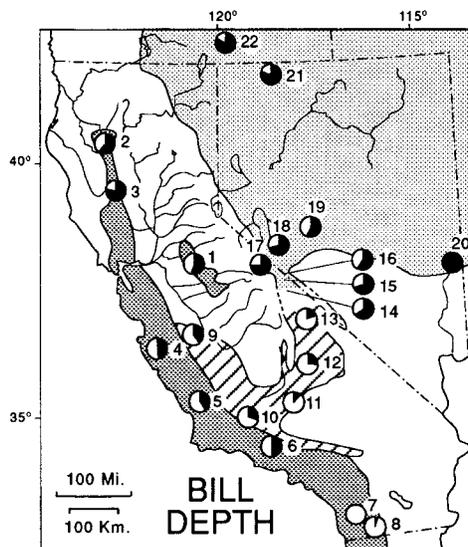
FIGURE 3.   SS-STP applied to population variation in bill length (upper) and bill depth (lower) in *A. belli*.

Desert birds were intermediate in bill width. Great Basin populations, starting at Chalfant Valley and continuing to Plush, Oregon, had the widest bills. The combined character, length of tarsus plus length of middle toe, repeated the broad trends seen in other features (Fig. 4, lower). Small to medium values occurred along the coast and through the southwestern interior of California to as far as Squaw Flat and large values began at Chalfant Valley and continued eastward and northward across the Great Basin. Only

Pioche, with an intermediate value, deviated from the general trend of large size of tarsus plus toe in far interior populations.

The final univariate character examined, cube root of body mass, also demonstrated the clear geographic pattern seen repeatedly in other features (Fig. 5): coastal populations were lightest, San Joaquin Valley and northern Mohave Desert populations were intermediate in body mass, and Great Basin birds were heaviest. The most pronounced geographic break occurred between

Sacatone Spring (8)	3.56
Barona Mesa (7)	3.64
Pozo (5)	3.67
Jawbone Canyon (11)	3.68
Carrizo Plains (10)	3.71
Monterey (4)	3.75
Beegum (2)	3.77
Castaic (6)	3.77
Coso Junction (12)	3.78
Panoche Hills (9)	3.79
Squaw Flat (13)	3.81
Coulterville (1)	3.89
Lake Co. (3)	3.91
Benton Valley (15)	4.07
Rattlesnake (18)	4.14
Chalfant Valley (14)	4.14
Mono (17)	4.15
Pioche (20)	4.18
Denio (21)	4.22
Queen Valley (16)	4.32
Plush (22)	4.33
Paradise Range (19)	4.38
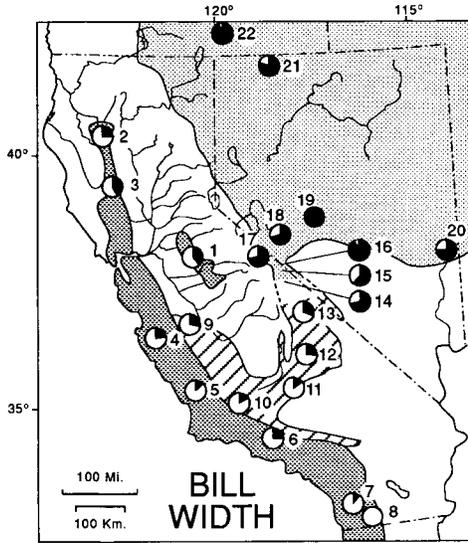
Beegum (2)	32.9
Sacatone Spring (8)	33.1
Monterey (4)	33.3
Pozo (5)	33.4
Barona Mesa (7)	33.4
Squaw Flat (13)	33.5
Castaic (6)	33.6
Coso Junction (12)	33.7
Lake Co. (3)	33.7
Jawbone Canyon (11)	33.7
Coulterville (1)	33.8
Panoche Hills (9)	34.3
Carrizo Plains (10)	34.4
Pioche (20)	34.5
Denio (21)	35.1
Rattlesnake Flat (18)	35.1
Queen Valley (16)	35.1
Chalfant Valley (14)	35.3
Paradise Range (19)	35.4
Benton Valley (15)	35.5
Mono (17)	35.6
Plush (22)	35.9
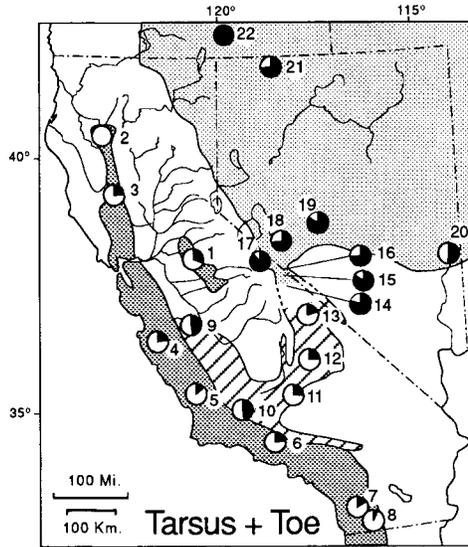
FIGURE 4.   SS-STP applied to population variation in bill width (upper) and length of tarsus plus length of middle toe (lower) in *A. belli*.

Squaw Flat and Chalfant Valley, repeating a pattern seen in several other morphologic features.

MULTIVARIATE ANALYSIS OF SIZE

The UPGMA dendrogram (Fig. 6), based on Euclidean distances among populations (see Methods), identified two major clusters: populations 1 through 13 separated unambiguously from populations 14 through 22. The first cluster was further subdivided, but by a short branch, into populations 1 through 6 and 7 through 13. In the latter group, populations 7 and 8, the two from San Diego County, allied as a sister group to southwestern interior populations 9 through 13. Because the branch defining this subdivision is short, a relationship of populations 7 and 8 to other coastal populations, 1 through 5, is almost equally probable.

Factor loadings for the first principal component axis were all high, ranging from 0.672 for bill depth to 0.972 for bill length (Table 1). On this basis, we regard PC1 as a size axis. Along

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Sacatone Spring (8)	2.435
Barona Mesa (7)	2.451
Coulterville (1)	2.456
Beegum (2)	2.464
Monterey (4)	2.467
Pozo (5)	2.474
Lake Co. (3)	2.478
Castaic (6)	2.496
Panoche Hills (9)	2.526
Jawbone Canyon (11)	2.545
Carrizo Plain (10)	2.548
Squaw Flat (13)	2.556
Coso Junction (12)	2.562
Benton Valley (15)	2.609
Queen Valley (16)	2.619
Chalfant Valley (14)	2.626
Rattlesnake Flat (18)	2.630
Plush (22)	2.638
Paradise Range (19)	2.652
Mono (17)	2.655
Denio (21)	2.656
Pioche (20)	2.656
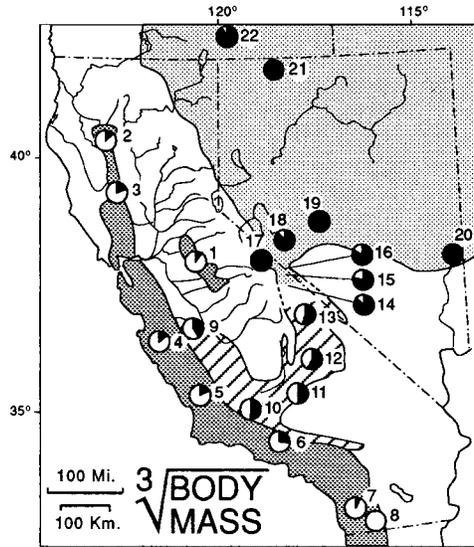
FIGURE 5.   SS-STP applied to population variation in cube root of body mass in *A. belli*.

PC2, only bill depth (-0.729) had a high loading. This axis may reflect a shape component, although as Bookstein (1989) and Sundberg (1989) note, axes other than PC1 can also contain some influence of size.

Ordination with multidimensional scaling (Fig. 7) clearly defined two major groupings among populations along Dimension I. All coastal and Mohavean populations (1 through 13) clustered separately from populations positioned from the north end of the White Mountains (14 through 16) and the Mono Region (17) across the interior of the Great Basin (18 through 22). The tightness of the cluster shown by the latter nine populations is impressive. In contrast, populations 1 through 13 form a much looser group. Populations 3, and 7 plus 8, the latter two from San Diego County, and 9 through 13, the populations from the San Joaquin Valley and northern Mohave Desert, are located peripherally to the main cluster. The low stress value (0.020) fell within the "excellent" range (Rohlf 1988), indicating that the MDS plot faithfully represented the original Euclidean distance matrix.

The minimum spanning tree superimposed on the MDS plot linked pairs of populations that are morphologically most similar (Fig. 7). Importantly, none of the population connections on this tree violated the two major clusters identified earlier. Furthermore, this tree clearly demonstrated that populations of *A. b. canescens* in the San Joaquin Valley and northern Mohave Desert (9 through 13) formed a cohesive subcluster on the basis of morphology.

GENETIC RESULTS

VARIATION AMONG LOCI AND HETEROZYGOSITY

Of the 41 loci scored, 17 (41.5%) were polymorphic. Twenty-four loci were monomorphic: AB-1, 2, 3, 4; ACON-2; ALD-1, 2; CK-1; GAPDH; GDA; G-6-PDH; GLUD; GOT-1, 2; GPT; ICD-2; LAP; LDH-2 (h); MDH-1, 2; PGM-2; SDH; SOD-1, 2.

Levels of genetic variability within populations of the *Amphispiza belli* complex are listed in Table 2. Observed heterozygosity ranged from 0.030 (Pozo) to 0.055 (Queen Valley). Average $H_{obs.}$ was 0.042. Percentage of polymorphic loci ranged from 14.63% (Pozo) to 34.15% (Benton Valley), with a mean value of 22.62%. Mean number of alleles per locus ranged from 1.17 (Monterey and Pozo) to 1.44 (Benton Valley), with an average of 1.29. Mean $H_{obs.}$ for *A. b. belli* (0.037) did not differ significantly (Kruskal-Wallis $T = 1.050$; $P = 0.306$) from that for *A. b. canescens* (0.040); however, the value for *A. b. nevadensis* (0.047) was significantly greater than that of either *A. b. belli* ($T = 7.521$; $P = 0.006$) or *A. b. canescens* ($T = 4.551$; $P = 0.033$). Sample size was uncorrelated ($P > 0.05$) with either mean observed heterozygosity ($r = 0.191$) or percent-
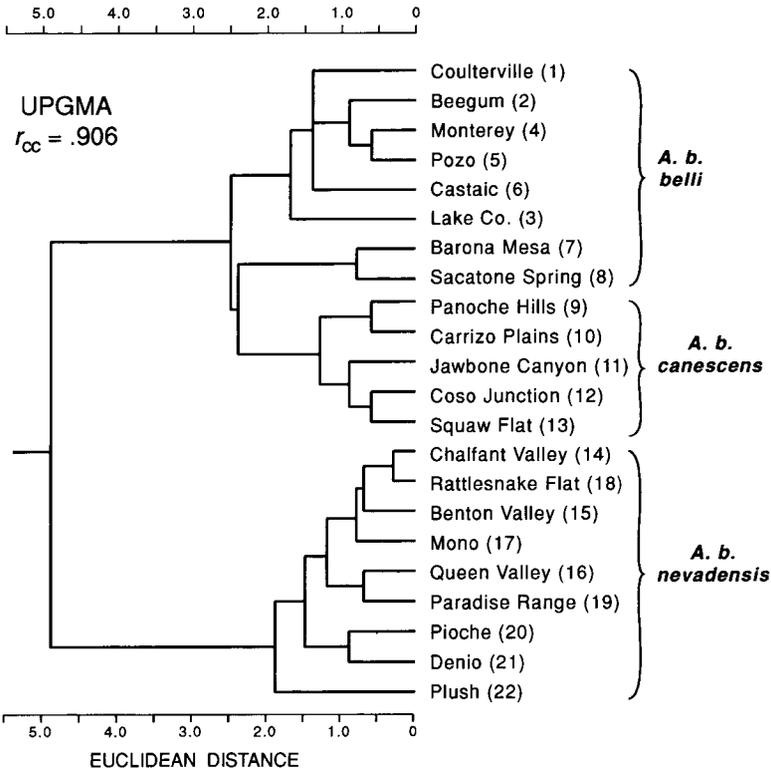
FIGURE 6. UPGMA dendrogram depicting the relationships of populations based on an analysis of Euclidean distances among variance standardized means. The high cophenetic coefficient (0.906) indicates very good agreement between the original data matrix and the dendrogram.

age of polymorphic loci ($r = 0.393$), but was significantly correlated with mean number of alleles per locus ($r = 0.471$; $P < 0.05$).

GEOGRAPHIC TRENDS IN ALLELIC FREQUENCY AND INTERSPECIFIC DIFFERENCES

A clear geographic pattern in the frequency of alleles occurred at the Pgm-1 locus (Table 3). In populations 1 through 13, Pgm-1c fluctuated from 80.6–100%, and appeared to be either fixed or nearing fixation. Starting with population 14 and continuing through the interior series to 22, in contrast, values for Pgm-1c varied between 36.1 and 63.3%. Nine populations had unique ("private") alleles. These were distributed randomly across the range of the species with the exception that Paradise Range and Benton Valley had unique alleles at two and three loci, respectively. The outgroup, *A. bilineata*, showed major allelic frequency differences from *A. belli* at ADA, EAP, LA-1, LDH, LGG, and 6-PGD (Table 3).

GENETIC DISTANCES

Nei's (1978) and Rogers' (1972) genetic distances were calculated for each of 276 pairwise comparisons of samples (Table 4). Intrataxon Nei's distances were low: $\bar{D} = 0.0019 \pm 0.00040$ in *A. b. belli*, $\bar{D} = 0.0015 \pm 0.00034$ in *A. b. canescens*, and $\bar{D} = 0.0007 \pm 0.00011$ in *A. b. nevadensis*.

TABLE 1. Factor loadings on the first three principal components of variation in six linear characters and cube root of body mass in male Sage Sparrows.

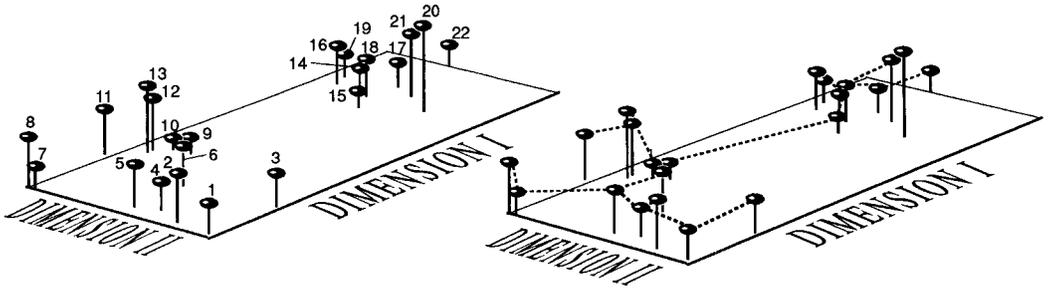|	Principal component		
	1	2	3
Wing length	0.962	0.188	0.113
Tail length	0.932	0.304	−0.079
Bill length	0.972	0.092	0.057
Bill depth	0.672	−0.729	0.038
Bill width	0.935	−0.216	−0.089
Tarsus + middle toe	0.956	0.054	−0.249
Cube root body mass	0.963	0.090	0.213

FIGURE 7. Multidimensional scaling plot with minimum spanning tree (dashed lines). See Figure 1 for geographic location of the 22 samples. Final stress value after 43 iterations = 0.020.

Mean values for Nei's D between populations representing different subspecies were higher: $\bar{D}$ = 0.0027 ± 0.00042 between *A. b. belli* and *A. b. canescens*, $\bar{D}$ = 0.0056 ± 0.00038 between *A. b. canescens* and *A. b. nevadensis*, and $\bar{D}$ = 0.0077 ± 0.00041 between *A. b. belli* and *A. b. nevadensis*. These values suggest that populations of the sedentary form *A. b. belli* are more fragmented than are those of the migratory form *A. b. nevadensis*, and that *A. b. canescens* is intermediate in this respect. Furthermore, intrataxon comparisons indicated that *A. b. canescens*, although geographically situated between *A. b. belli* and *A. b. nevadensis*, is genetically closer to the more coastal form *A. b. belli* than to the interior form *A. b. nevadensis*.

Nei's distances were highest for the 44 interspecific comparisons of *A. belli* with *A. bilineata*, where $\bar{D}$ = 0.1357 ± 0.00299. Unexpectedly, Nei's D was higher when *A. belli* was compared with *A. bilineata deserticola* ($\bar{D}$ = 0.1548 ± 0.00094) than when compared with *A. b. opuntia* ($\bar{D}$ = 0.1165 ± 0.00091), a difference that is highly significant (t = 29.366, P < 0.001).

GENETIC POPULATION STRUCTURE AND GENE FLOW

Wright's F_{st} statistic for all 22 populations of *A. belli* was 0.112. Thus, 11.2% of the genetic variance present was distributed among populations, which points to significant fragmentation. Calculation of F_{st} in population subsets can also be illuminating. For example, F_{st} averaged 0.071 within the sedentary *A. b. belli*, whereas in the migratory forms *A. b. canescens* and *A. b. nevadensis*, F_{st} values were 0.047 and 0.037, respectively. The latter two populations were genetically less fragmented, reinforcing the intuitive view that migratory behavior mixes populations.

When subspecies were combined into subsets, some high F_{st} values resulted: *A. b. belli* plus *A. b. nevadensis*, F_{st} = 0.115; *A. b. canescens* plus *A. b. nevadensis*, 0.083; and *A. b. belli* plus *A. b. canescens*, 0.078.

Using Wright's formula to calculate Nm from F_{st}, Nm equalled 1.98 over all populations. Private alleles also allow estimation of gene flow (Slatkin 1985a, 1985b; Table 5). Estimated number of immigrants per generation (Nm_c) varied from 0.4 (Chalfant Valley) to 18.3 (Pioche and Denio). Coulterville, with 12 immigrants per generation, had a surprisingly high value for a sedentary population, and Chalfant Valley, Queen Valley, and Paradise Range, with Nm_c ranging from 0.4 to 3.3, had unexpectedly low values for migratory populations. The unusually high frequency (0.18) of the single private allele at Chalfant Valley is unexplained. The influence of a single, isolated, or otherwise deviant sample (such as Chalfant Valley) on the estimate of Nm can be assessed by computing $p(1)$ for different subsamples of the entire data set (Slatkin 1985). The right half of Table 5 gives estimated values of Nm_c in eight of the nine total samples after the successive removal of different single samples. The value of Nm_c of 4.33 is similar to those of all other samples when any sample except Chalfant Valley was excluded. When the sample from Chalfant Valley was excluded, however, the estimate of Nm_c increased to nearly 6.9, a value 62.7% greater.

UPGMA CLUSTER ANALYSIS

To enable visual assessment of relationships, a phenogram was produced from Rogers' genetic distances for all 22 populations of *A. belli* plus two populations of *A. bilineata* as a composite outgroup (Fig. 8). The Black-throated Sparrow

TABLE 2.   Genetic variability measures for 22 samples representing three taxa of the *Amphispiza belli* complex.

Sample number and name		n	$H_{obs.} \pm SE$	Percent poly-morphic loci	Mean no. alleles
1	Coulterville	16	0.034 ± 0.013	19.51	1.27
2	Beegum	19	0.033 ± 0.015	17.07	1.20
3	Lake Co.	17	0.040 ± 0.014	21.95	1.29
4	Monterey	12	0.033 ± 0.017	17.07	1.17
5	Pozo	13	0.030 ± 0.013	14.63	1.17
6	Castaic	14	0.037 ± 0.014	21.95	1.27
7	Barona Mesa	18	0.045 ± 0.015	21.95	1.29
8	Sacatone Spring	9	0.043 ± 0.018	19.51	1.24
9	Panoche Hills	17	0.043 ± 0.017	21.95	1.24
10	Carrizo Plains	20	0.044 ± 0.016	21.95	1.32
11	Jawbone Canyon	15	0.036 ± 0.015	17.07	1.24
12	Coso Junction	18	0.042 ± 0.013	26.83	1.34
13	Squaw Flat	15	0.034 ± 0.018	19.51	1.20
14	Chalfant Valley	14	0.044 ± 0.018	21.95	1.29
15	Benton Valley	19	0.053 ± 0.017	34.15	1.44
16	Queen Valley	17	0.055 ± 0.017	26.83	1.39
17	Mono	16	0.049 ± 0.018	24.39	1.34
18	Rattlesnake Flat	15	0.049 ± 0.018	29.27	1.37
19	Paradise Range	15	0.052 ± 0.017	26.83	1.34
20	Pioche	20	0.038 ± 0.014	24.39	1.29
21	Denio	20	0.039 ± 0.014	21.95	1.34
22	Plush	18	0.045 ± 0.016	26.83	1.32
	Mean	16.2	0.0417	22.615	1.289

segregated at a high distance from the Sage Sparrow. Surprisingly, the two populations of *A. bilineata* diverged more strongly than anticipated, suggesting the need for further genetic study of that species. Within *A. belli,* two large clusters are evident, one containing all populations attributed to *A. b. belli* and *A. b. canescens* and the other comprised of all Great Basin populations (*A. b. nevadensis*) from Chalfant Valley northward to Oregon. *A. b. canescens* is clearly more similar genetically to *A. b. belli* than to *A. b. nevadensis.* Within each major cluster of *A. belli,* branch lengths separating populations are short and therefore relatively unreliable as a basis for interpreting relationships. Nevertheless, it is worth noting that Coso Junction and Squaw Flat form a sister group to other populations in the Coast Range-San Joaquin Valley-Northern Mojave Desert cluster and that the Paradise Range sample separated from the remainder of the Great Basin group.

MANTEL TEST

A Mantel test (Mantel 1967) demonstrated highly significant congruence between morphologic and genetic patterns of variation. In a comparison of a matrix of Euclidean distances derived from morphometric characters with a matrix of Rogers' genetic distances, $r = 0.6858$, $t = 10.209$, and $P = 1.000$. A similar comparison of matrices of Euclidean and Nei's genetic distances yielded nearly identical values: $r = 0.6836$, $t = 10.038$, and $P = 1.000$. Probabilities are significant in Mantel tests when close to either 0 or 1.

DISCUSSION AND CONCLUSIONS

GENETIC VARIATION, POPULATION STRUCTURE, AND GENE FLOW

The mean level of observed heterozygosity in the Sage Sparrow, 0.042, was lower than the value reported by Barrowclough (1983) as typical of other species of birds (0.053). However, reports of low $\bar{H}_{obs.}$ in more recent literature (e.g., Baker and Strauch 1988, Johnson and Marten 1988, and Johnson et al. 1989) suggest that the average of 5.3% could be adjusted downward with the inclusion of additional species. Despite this possibility, it is worth noting that *A. b. nevadensis* had significantly higher levels of observed heterozygosity than either *A. b. belli* or *A. b. canescens.* We have no reason to suspect that either of the latter forms passed through population bottlenecks during their evolutionary history (Nei et al. 1975), an explanation commonly invoked

TABLE 3. Allelic frequencies at 17 variable loci in 22 populations of *Amphispiza belli* and two populations of *A. bilineata*.

Locus (EC no.)	Allele	1 Coulter-ville	2 Beegum	3 Lake Co.	4 Mon-terey	5 Pozo	6 Castaic	7 Barona Mesa	8 Sacatone Spring	9 Panoche Hills	10 Carrizo Plains	11 Jawbone Canyon
ACON	a											
(4.2.1.3)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA	a	0.031		0.029								0.033
(3.4.4.4)	b		0.026									
	c	0.969	0.921	0.941	0.875	0.923	0.893	0.972	1.000	0.971	0.875	0.833
	d		0.053	0.029	0.125	0.077	0.107	0.028		0.029	0.125	0.133
	e											
ADH	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.941	1.000	1.000
(1.1.1.1)	b									0.059		
α-GPD	a							0.056				
(1.1.1.8)	b			0.059	0.042		0.036		0.056			
	c	0.969	1.000	0.941	0.958	1.000	0.929	0.944	0.944	1.000	0.975	1.000
	d	0.031					0.036					
	e										0.025	
	f											
CK-2	a											
(2.7.3.2)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EAP	a											
(3.1.3.2)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c											
EST-4	a			0.029				0.056				
(3.1.1.1)	b	0.938	0.868	0.912	1.000	1.000	0.893	0.917	0.944	0.971	0.950	1.000
	c	0.063	0.132	0.059			0.107	0.028	0.056	0.029	0.050	
GPI	a											
(5.3.1.9)	b	0.094	0.158	0.206		0.154	0.107	0.194	0.056	0.265	0.250	0.133
	c										0.025	0.100
	d	0.906	0.842	0.794	1.000	0.846	0.893	0.806	0.944	0.735	0.725	0.767
	e											
ICD-1	a											0.033
(1.1.1.42)	b	1.000	1.000	1.000	0.958	1.000	1.000	1.000	1.000	1.000	0.975	0.967
	c										0.025	
	d				0.042							
LA-1	a											
(3.4.11)	b											
	c											
	d											
	e	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LA-2	a	0.031	0.026	0.088	0.042	0.038	0.071	0.111		0.088	0.125	0.100
(3.4.11)	b	0.844	0.974	0.882	0.958	0.846	0.893	0.833	0.944	0.912	0.825	0.900
	c	0.125		0.029		0.115	0.036	0.056	0.056		0.050	
	d											
LDH	a											
(1.1.1.27)	b											
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LGG	a											
(3.4.11)	b			0.029		0.038		0.139	0.111			
	c	1.000	1.000	0.971	1.000	0.962	0.964	0.861	0.833	1.000	1.000	1.000
	d											
	e						0.036		0.056			
MPI	a											
(5.3.1.8)	b	0.063	0.553	0.588	0.500	0.308	0.286	0.111	0.278	0.353	0.250	0.100
	c											
	d	0.938	0.447	0.412	0.500	0.692	0.714	0.889	0.722	0.647	0.750	0.900

TABLE 3. Continued.

12 Coso Junction	13 Squaw Flat	14 Chalfant Valley	15 Benton Valley	16 Queen Valley	17 Mono	18 Rattle- snake Flat	19 Paradise Range	20 Pioche	21 Denio	22 Plush	A. bilineata (Nevada)	A. bilineata (Okla.)
						0.067	0.133	0.125				
1.000	1.000	1.000	1.000	1.000	1.000	0.933	0.867	0.875	1.000	1.000	1.000	1.000
0.222		0.036		0.088			0.100	0.075				
		0.036		0.059	0.031	0.033						
0.778	0.967	0.929	1.000	0.853	0.906	0.967	0.833	0.925	1.000	0.972		0.333
	0.033				0.063					0.028		
							0.067				1.000	0.667
0.972	1.000	1.000	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.028			0.026									
					0.031			0.050				
0.028												
0.889	0.900	0.964	0.974	0.941	0.938	0.833	0.967	0.875	0.875	0.944	1.000	1.000
0.083	0.100	0.036		0.059	0.031	0.167	0.033	0.075	0.125	0.056		
			0.026									
			0.026									
1.000	1.000	1.000	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
			0.026									
1.000	1.000	1.000	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000		
											1.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
							0.033		0.025			
0.250	0.333	0.143	0.105	0.059	0.188	0.267	0.100	0.050	0.250	0.167		0.167
			0.026									
0.750	0.667	0.857	0.842	0.912	0.813	0.733	0.867	0.950	0.725	0.806	1.000	0.833
			0.026	0.029						0.028		
0.028	0.033		0.026			0.033						
0.972	0.967	1.000	0.974	1.000	1.000	0.967	1.000	1.000	1.000	0.972	1.000	1.000
										0.028		
											0.250	0.167
									0.025		0.750	0.500
		0.036	0.053	0.029	0.031	0.067	0.100	0.025		0.056		
												0.333
1.000	1.000	0.964	0.947	0.971	0.969	0.933	0.900	0.975	0.975	0.944		
0.028	0.067	0.071	0.026			0.033	0.100	0.100	0.025	0.056		0.333
0.917	0.933	0.929	0.974	0.941	0.938	0.933	0.900	0.900	0.950	0.944	1.000	0.667
0.056				0.059	0.063	0.033			0.025			
											1.000	1.000
			0.053	0.059	0.031							
1.000	1.000	1.000	0.947	0.941	0.969	1.000	1.000	1.000	1.000	1.000		
				0.059	0.031		0.033		0.025	0.028		
		0.036	0.026	0.088	0.063	0.033			0.050	0.056		
0.944	1.000	0.964	0.842	0.794	0.875	0.900	0.967	0.950	0.825	0.917		0.167
0.056			0.132	0.059	0.031	0.067		0.050	0.100			0.333
											1.000	0.500
						0.067						
0.083		0.071	0.132	0.147	0.094	0.033	0.200	0.100	0.150	0.167		0.167
		0.179										
0.917	1.000	0.750	0.868	0.853	0.906	0.967	0.733	0.900	0.850	0.833	1.000	0.833

TABLE 3.   Continued.

Locus (EC no.)	Allele	1 Coulter-ville	2 Beegum	3 Lake Co.	4 Mon-terey	5 Pozo	6 Castaic	7 Barona Mesa	8 Sacatone Spring	9 Panoche Hills	10 Carrizo Plains	11 Jawbone Canyon
NP	a									0.029		
(2.4.2.1)	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.971	1.000	1.000
	c											
6-PGD	a											0.033
(1.1.1.44)	b					0.038		0.083	0.056	0.059	0.050	
	c	0.031										
	d	0.031										
	e	0.781	0.921	0.853	0.958	0.962	0.964	0.889	0.944	0.853	0.900	0.967
	f											
	g	0.156	0.079	0.147	0.042		0.036	0.028		0.088	0.050	
PGM-1	a								0.056			
(2.7.5.1)	b										0.025	0.033
	c	0.875	0.974	0.941	0.958	1.000	0.964	0.917	0.833	0.941	0.925	0.933
	d	0.125	0.026	0.059	0.042		0.036	0.083	0.111	0.059	0.050	0.033
	e											

to explain reduced genetic variability when the true reason is unknown.

Several lines of analysis indicated that the largely sedentary form *A. b. belli* has a more fragmented genetic population structure than the highly migratory form *A. b. nevadensis*. This inverse relationship between genetic population fragmentation and relative development of migratory behavior is clearly seen in the intrataxon comparisons of Nei's *D*, in which populations of

TABLE 4.   Matrix of genetic distances between 22 samples of three taxa of the *Amphispiza belli* complex and two samples of two taxa of *A. bilineata*. Nei's (1978) *D*-values are above the diagonal, and Rogers' (1972) *D*-values are below the diagonal.

	1	2	3	4	5	6	7	8	9	10
1. Coulterville	—	0.006	0.007	0.006	0.002	0.001	0	0.001	0.002	0.001
2. Beegum	0.026	—	0	0	0.001	0.001	0.005	0.002	0.001	0.002
3. Lake Co., Calif.	0.024	0.011	—	0.001	0.002	0.002	0.005	0.002	0.001	0.002
4. Monterey	0.028	0.014	0.018	—	0.001	0.001	0.005	0.001	0.002	0.003
5. Pozo	0.020	0.016	0.019	0.017	—	0	0.001	0	0	0
6. Castaic	0.019	0.015	0.018	0.015	0.011	—	0.001	0	0	0
7. Barona Mesa	0.016	0.026	0.022	0.030	0.018	0.019	—	0	0.001	0
8. Sacatone Spring	0.019	0.024	0.024	0.021	0.017	0.015	0.018	—	0.001	0.001
9. Panoche Hills	0.022	0.017	0.016	0.021	0.015	0.017	0.019	0.022	—	0
10. Carizzo Plains	0.020	0.020	0.019	0.020	0.014	0.013	0.016	0.021	0.013	—
11. Jawbone Canyon	0.019	0.024	0.026	0.021	0.016	0.017	0.017	0.025	0.020	0.013
12. Coso Junction	0.023	0.034	0.032	0.032	0.026	0.026	0.023	0.027	0.025	0.023
13. Squaw Flat	0.019	0.032	0.029	0.032	0.027	0.028	0.022	0.029	0.021	0.021
14. Chalfant Valley	0.025	0.031	0.031	0.031	0.024	0.026	0.025	0.025	0.028	0.027
15. Benton Valley	0.035	0.043	0.046	0.042	0.037	0.038	0.033	0.032	0.038	0.039
16. Queen Valley	0.032	0.042	0.043	0.038	0.034	0.034	0.031	0.028	0.039	0.037
17. Mono	0.030	0.037	0.038	0.037	0.029	0.031	0.025	0.029	0.035	0.031
18. Rattlesnake Flat	0.033	0.044	0.043	0.043	0.037	0.037	0.031	0.035	0.035	0.035
19. Paradise Range	0.031	0.035	0.035	0.032	0.027	0.027	0.032	0.028	0.030	0.028
20. Pioche	0.030	0.043	0.042	0.037	0.034	0.033	0.031	0.030	0.039	0.036
21. Denio	0.029	0.035	0.034	0.036	0.028	0.030	0.023	0.023	0.027	0.027
22. Plush	0.032	0.038	0.038	0.037	0.029	0.031	0.027	0.028	0.032	0.031
23. *A. bilineata* (Nev.)	0.154	0.164	0.168	0.158	0.156	0.158	0.156	0.156	0.163	0.161
24. *A. bilineata* (Okla.)	0.139	0.145	0.145	0.146	0.133	0.139	0.133	0.141	0.140	0.136

TABLE 3. Continued.

12 Coso Junction	13 Squaw Flat	14 Chalfant Valley	15 Benton Valley	16 Queen Valley	17 Mono	18 Rattle-snake Flat	19 Paradise Range	20 Pioche	21 Denio	22 Plush	A. bilineata (Nevada)	A. bilineata (Okla.)
0.028			0.053	0.118		0.033	0.033		0.025			
0.972	0.967	1.000	0.947	0.882	1.000	0.967	0.967	1.000	0.975	0.972	1.000	1.000
	0.033											
0.028							0.033					0.167
		0.036						0.025	0.025	0.028	1.000	0.833
0.028		0.036	0.053	0.029								
0.944	0.867	0.929	0.921	0.912	0.969	0.967	0.967	0.975	0.950	0.972		
				0.059								
	0.133		0.026		0.031	0.033			0.025			
						0.033						
0.806	0.833	0.536	0.368	0.529	0.406	0.500	0.633	0.400	0.625	0.361	1.000	1.000
0.194	0.167	0.464	0.632	0.471	0.594	0.467	0.367	0.575	0.375	0.639		
								0.025				

A. b. belli are genetically more distinctive than are those of *A. b. nevadensis*. Again, Wright's F_{st} points to greater genetic uniformity in the migratory *A. b. nevadensis*. The weakly migratory *A. b. canescens* is intermediate in this respect, although values for both Nei's *D* and F_{st} are closer to *A. b. nevadensis*.

Barrowclough and Johnson (1988) concluded that populations of North American temperate-zone species of birds, characterized by moderate to large effective population sizes and significant levels of gene flow, exhibit reduced intraspecific differentiation relative to other vertebrate groups. Population genetic information offered here for

TABLE 4. Continued.

11	12	13	14	15	16	17	18	19	20	21	22	23	24
0.001	0.002	0.001	0.001	0.007	0.004	0.006	0.005	0.003	0.006	0.003	0.008	0.150	0.113
0.005	0.007	0.009	0.009	0.014	0.010	0.013	0.013	0.006	0.014	0.008	0.013	0.160	0.120
0.006	0.007	0.009	0.009	0.014	0.010	0.013	0.013	0.006	0.014	0.008	0.013	0.161	0.118
0.004	0.007	0.009	0.008	0.013	0.008	0.012	0.012	0.005	0.012	0.008	0.012	0.157	0.118
0.001	0.003	0.004	0.006	0.011	0.007	0.009	0.008	0.003	0.010	0.005	0.010	0.153	0.113
0.001	0.002	0.003	0.005	0.010	0.006	0.008	0.007	0.003	0.009	0.004	0.009	0.152	0.114
0	0.001	0.001	0.004	0.008	0.004	0.006	0.005	0.003	0.007	0.002	0.008	0.148	0.109
0.002	0.002	0.004	0.003	0.006	0.003	0.005	0.005	0.002	0.006	0.002	0.006	0.148	0.112
0.002	0.003	0.003	0.005	0.010	0.007	0.009	0.007	0.003	0.010	0.004	0.009	0.155	0.113
0	0.002	0.002	0.005	0.010	0.006	0.008	0.006	0.003	0.009	0.003	0.009	0.153	0.112
—	0.001	0.001	0.004	0.009	0.005	0.007	0.005	0.003	0.008	0.004	0.009	0.151	0.114
0.018	—	0.001	0.003	0.006	0.003	0.004	0.002	0.002	0.005	0.001	0.006	0.151	0.114
0.020	0.017	—	0.004	0.007	0.005	0.005	0.002	0.004	0.007	0.001	0.006	0.155	0.116
0.025	0.025	0.026	—	0.001	0	0	0.001	0	0.001	0.001	0	0.156	0.119
0.034	0.031	0.035	0.022	—	0	0	0.001	0.002	0.001	0.001	0	0.158	0.121
0.033	0.028	0.035	0.019	0.020	—	0	0.001	0.001	0.001	0.001	0.001	0.150	0.115
0.027	0.025	0.029	0.016	0.016	0.017	—	0	0.002	0	0.001	0	0.158	0.122
0.031	0.023	0.022	0.021	0.024	0.025	0.017	—	0.001	0.001	0	0	0.159	0.121
0.026	0.026	0.033	0.018	0.030	0.024	0.026	0.025	—	0.001	0.001	0.002	0.152	0.115
0.029	0.028	0.033	0.018	0.023	0.022	0.016	0.020	0.019	—	0.002	0	0.161	0.124
0.027	0.020	0.021	0.019	0.021	0.020	0.018	0.014	0.023	0.024	—	0.001	0.155	0.116
0.029	0.028	0.028	0.015	0.014	0.021	0.011	0.019	0.023	0.017	0.017	—	0.163	0.125
0.154	0.160	0.160	0.164	0.166	0.161	0.164	0.169	0.164	0.168	0.164	0.169	—	0.005
0.135	0.142	0.147	0.146	0.149	0.145	0.147	0.154	0.144	0.153	0.144	0.149	0.047	—

TABLE 5.   Number and average frequency of private alleles and estimates of gene flow in populations of *Amphispiza belli.* See Table 2 for sample size of each population. Average sample size was 16.23. Only populations with private alleles were included in the analysis.

	Single populations			Combined populations (named population excluded)[a]		
	No. private alleles	$p(1)$	Nm_c[b]	No. private alleles	$p(1)$	Nm_c
Sample location						
Coulterville	1	0.031	11.94	11	0.054	3.98
Monterey	1	0.042	6.53	11	0.053	4.12
Sacatone Spring	1	0.056	3.70	11	0.052	4.28
Chalfant Valley	1	0.179	0.371	11	0.041	6.86
Benton Valley	3	0.026	16.90	9	0.061	3.13
Queen Valley	1	0.059	3.34	11	0.052	4.29
Paradise Range	2	0.067	2.59	10	0.050	4.63
Pioche	1	0.025	18.27	11	0.055	3.83
Denio	1	0.025	18.27	11	0.055	3.83
All samples				10.7	0.053	4.33

[a] For example, 11 private alleles occurred at an average frequency of 0.054 in the 8 populations excluding Coulterville.
[b] $Nm_c = Nm$ corrected for sample size.

the Sage Sparrow complex broadly agrees with this generalization. Although the overall estimate of Wright's F_{st} is high (0.112) for intraspecifically differentiated taxa, the direct estimate of gene flow rate obtained both from F_{st} (Wright 1951) and from the spatial distribution of private alleles (Slatkin 1985a, 1985b), at approximately 2–4 immigrants per generation, is comparable with other recent figures (Zink and Remsen 1986; Rockwell and Barrowclough 1987; Johnson and Marten 1988, 1991).

CONGRUENCE OF MORPHOMETRIC AND GENETIC PATTERNS

The results of the Mantel test statistically demonstrated the striking concordance of morphometric and genetic patterns across geography in the Sage Sparrow complex. Furthermore, this broad-scale congruence is completely corroborated by an analysis of mtDNA base sequences at the cytochrome b locus (Johnson and Cicero, in press). These results support three major conclusions: (1) *A. b. belli, A. b. canescens,* and *A. b. nevadensis* are each distinctive, integrated evolutionary units; (2) *A. b. belli* and *A. b. canescens* are each other's closest relatives; and (3) populations at the northern end of the White Mountains show no intergradation with *A. b. canescens* as reported in the literature (Grinnell and Miller 1944:500). Three populations from that region examined in this study, Chalfant Valley, Benton Valley, and Queen Valley, are all typical *A. b. nevadensis.* Even the southernmost population (Chalfant Valley), which is closest to populations

of *A. b. canescens* and therefore might be likely to show intergradation with the latter form, shows the mtDNA sequence pattern of Rattlesnake Flat and Plush (*A. b. nevadensis*) rather than that of Panoche Hills (*A. b. canescens*).

TAXONOMIC AND EVOLUTIONARY STATUS OF POPULATIONS

Although most populations of the San Joaquin Valley and northern Mohave Desert (*A. b. canescens*) average significantly larger than those in the Coast Ranges and western slope of the Sierra Nevada (*A. b. belli*) in all characters studied here, it is not certain that a formal subspecies should be recognized on this basis alone. Despite only moderate size differentiation, however, overall coloration continues to be a valid criterion for their separation. Examination of hundreds of museum specimens, with due regard for season and degree of plumage wear, indicates that the two forms are 100% separable on color alone. Grinnell (1898, 1905) commented on the apparent lack of specimens intermediate between the two forms and, partly on this basis, argued for their specific status. Four decades later he altered his view (Grinnell and Miller 1944: 501) and reported "intergrades" from San Benito County, California, that were part of the collections of the Museum of Vertebrate Zoology. One of us (NKJ) has studied these specimens and visited the collection locality on San Benito Mountain (Johnson and Cicero 1985:12). According to the field notes of the collectors, the birds were in non-breeding condition and oc-
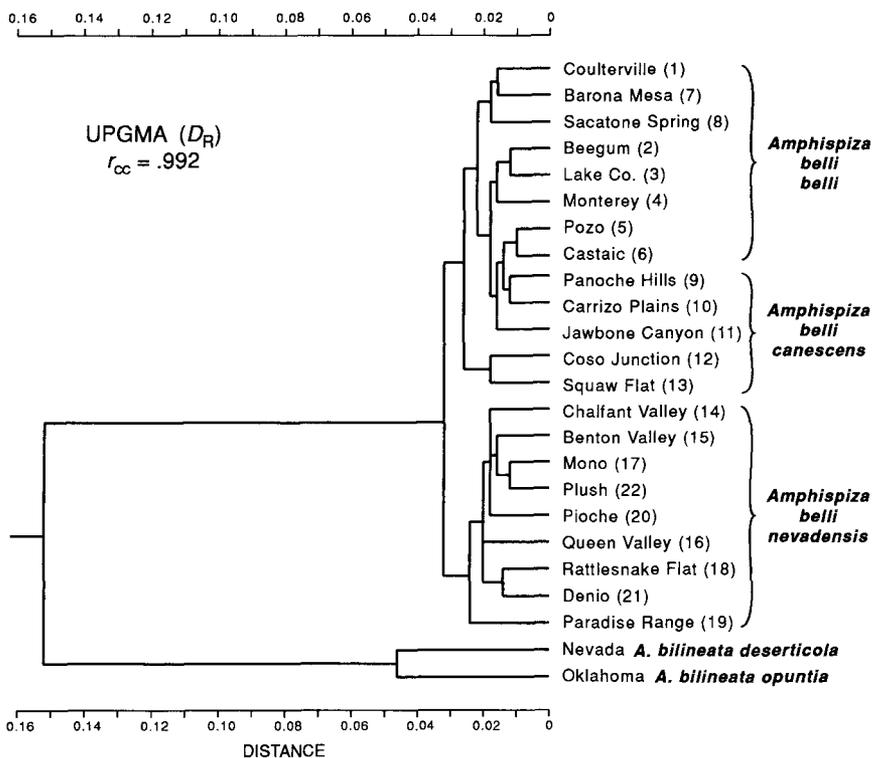
FIGURE 8. UPGMA cluster analysis of Rogers' (1972) genetic distances among 22 population samples of *Amphispiza belli* and two of *A. bilineata*. The very high cophenetic correlation coefficient (0.992) indicates excellent agreement between the original data matrix and the dendrogram.

curred in flocks. Thus, no evidence exists that this species nests on San Benito Mountain. In our opinion, these supposed examples of intergradation are instead typical *A. b. canescens* in fresh, post-breeding plumage acquired after an uphill migration from nesting localities in the adjacent lowlands of the San Joaquin Valley. A. H. Miller, who collected most of the specimens and interpreted them as intergrades in the account in Grinnell and Miller (1944), presumably judged them (incorrectly) to be on their nesting grounds. Because these specimens are in fresh plumage and are therefore more richly colored and slightly darker than worn nesting individuals of typical *A. b. canescens,* Miller evidently viewed the increased pigmentation as evidence for intermediacy with *A. b. belli.* An analysis of wing length of the 24 males in this series also confirms their status as typical *A. b. canescens.* In mean wing length, the San Benito County specimens do not differ significantly from the Panoche Hills series, the geographically nearest sample of breeding *A. b. canescens* ($t = 0.404$; $P > 0.5$).

However, the San Benito County birds are highly significantly different in wing length from the nearest sample of breeding *A. b. belli* from Monterey ($t = 4.296$; $P < 0.001$).

Two other issues are pertinent to a discussion of the taxonomic and evolutionary distinction of *A. b. belli* and *A. b. canescens.* First, despite the close geographic approach of their preferred breeding habitats, coastal chaparral and arid interior brushland, respectively, we are unaware of evidence that these vegetation types intermix in California, which would allow contact of the two forms of Sage Sparrow. Instead, where major stands of chamise and saltbush approach in southwestern California, they seem always to be separated by a gap, albeit narrow, of habitat inappropriate for either form. Chamise is prevalent on west-facing slopes or cooler mountain sites in the Coast Range. Saltbush, in contrast, flourishes on the warm and arid flats of interior San Joaquin Valley and locally approaches, but does not contact, chamise in washes that penetrate the foothills rising from the western side of the valley.

Second, post-nesting *A. b. canescens* move up-hill in late spring and early summer into the active breeding range of *A. b. belli.* Inexplicably, this mixing during the nesting season does not result in interbreeding. While males of *A. b. belli* actively sing, defend territories, and court females, adults of both sexes of *A. b. canescens* skulk and molt in nonbreeding condition *in the same thickets,* a phenomenon that NKJ has documented in the chamise brushland near Coulterville, on the west slope of the Sierra Nevada, and in the same habitat in the Pozo region of San Luis Obispo County. Thus, these two forms of sparrow act as if they are reproductively isolated. Intensive exploration of regions where their preferred shrub habitats approach but are not now known to intermix should be undertaken before firm conclusions on the absence of interbreeding can be reached.

The taxonomic and evolutionary status of *A. b. canescens* and *A. b. nevadensis* is also more complicated than previously believed. Their supposed intergradation in the vicinity of Benton, Mono County, California (Grinnell and Miller 1944:500), is not supported by the data of the present paper. Instead, populations in the vicinity of the north end of the White Mountains, including those at Benton and Chalfant Valley, are clearly *A. b. nevadensis* on both morphometric and genetic grounds. Therefore, if a region of contact and intergradation exists between these forms it must be situated somewhere in the 100 mile 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). Investigation of this zone should yield information on the possible biologic species status of these two strongly differentiated forms. One of us (NKJ) is currently studying this topic.

ACKNOWLEDGMENTS

LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds. 5th ed. American Ornithologists' Union, Washington, DC.

AMERICAN ORNITHOLOGISTS' UNION. 1983. Check-list of North American birds. 6th ed. American Ornithologists' Union, Washington, DC.

BAKER, A. J., AND J. G. STRAUCH, JR. 1988. Genetic variation and differentiation in shorebirds. Acta XIX Congressus Internationalis Ornithologici 2: 1639–1645.

BARROWCLOUGH, G. F. 1983. Biochemical studies of microevolutionary processes, p. 223–261. *In* A. H. Brush and G. A. Clark, Jr. [eds.], Perspectives in ornithology. Cambridge Univ. Press, Cambridge, England.

BARROWCLOUGH, G. F., AND N. K. JOHNSON. 1988. Genetic structure of North American birds. Acta XIX Congressus Internationalis Ornithologici 2: 1630–1638, 1669–1673.

BOOKSTEIN, F. L. 1989. "Size and shape": a comment on semantics. Syst. Zool. 38:173–180.

GABRIEL, K. R. 1964. A procedure for testing the homogeneity of all sets of means in analysis of variance. Biometrics 20:459–477.

GABRIEL, K. R., AND R. R. SOKAL. 1969. A new statistical approach to geographic variation analysis. Syst. Zool. 18:259–278.

GRINNELL, J. 1898. Rank of the sage sparrow. Auk 15:58–59.

GRINNELL, J. 1905. The California sage sparrow. Condor 7:18–19.

GRINNELL, J., AND A. H. MILLER. 1944. The distribution of the birds of California. Pac. Coast Avif. 27:1–608.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing, Amsterdam.

JOHNSON, N. K. 1980. Character variation and evolution of sibling species in the *Empidonax difficilis-flavescens* complex (Aves: Tyrannidae). Univ. Calif. Publ. Zool. 112:1–151.

JOHNSON, N. K., AND C. CICERO. 1985. The breeding avifauna of San Benito Mountain, California: evidence for change over one-half century. Western Birds 16:1–23.

JOHNSON, N. K., AND C. CICERO. In press. Mitochondrial DNA sequence variability in two species of sparrows of the genus *Amphispiza.* Proc. XXth International Ornithological Congress, Christchurch, New Zealand, 1990.

JOHNSON, N. K., AND J. A. MARTEN. 1988. Evolutionary genetics of flycatchers. II. Differentiation in the *Empidonax difficilis* complex. Auk 105:177–191.

JOHNSON, N. K., AND J. A. MARTEN. 1991. Evolu-

tionary genetics of flycatchers. III. Variation in *Empidonax hammondii* (Aves: Tyrannidae). Can. J. Zool. 69:232–238.

JOHNSON, N. K., J. A. MARTEN, AND C. J. RALPH. 1989. Genetic evidence for the origin and relationships of Hawaiian honeycreepers (Aves: Fringillidae). Condor 91:379–396.

JOHNSON, N. K., R. M. ZINK, G. F. BARROWCLOUGH, AND J. A. MARTEN. 1984. Suggested techniques for modern avian systematics. Wilson Bull. 96: 543–560.

MANTEL, N. 1967. The detection of disease clustering and a general regression approach. Cancer Res. 27: 209–220.

NEI, M. 1975. Molecular population genetics and evolution. North Holland Publishing, Amsterdam.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

ROCKWELL, R. F., AND G. F. BARROWCLOUGH. 1987. Gene flow and the genetic structure of populations, p. 223–255. *In* F. Cooke and P. A. Buckley [eds.], Avian genetics: a population and ecological approach. Academic Press, Orlando, FL.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Stud. Genet. 7: 145–153.

ROHLF, F. J. 1988. NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.50. Exeter Publ., Setauket, NY.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Texas Stud. Genet. 6: 49–90.

SLATKIN, M. 1981. Estimating levels of gene flow in natural populations. Genetics 99:323–335.

SLATKIN, M. 1985a. Rare alleles as indicators of gene flow. Evolution 39:53–65.

SLATKIN, M. 1985b. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393–430.

SLATKIN, M., AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry, the principles and practice of statistics in biological research. W. H. Freeman, San Francisco.

SUNDBERG, P. 1989. Shape and size-constrained principal components analysis. Syst. Zool. 38:166–168.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIO-SYS-1: a FORTRAN program for the comprehensive analysis of genetic data in population genetics and systematics. J. Hered. 72:281–283.

WRIGHT, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323–354.

WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19:395–420.

WRIGHT, S. 1978. Evolution and the genetics of natural populations. Vol. 4. Variability within and among natural populations. Univ. Chicago Press, Chicago.

YANG, S. Y., AND J. L. PATTON. 1981. Genic variability and differentiation in the Galapagos finches. Auk 98:230–242.

ZINK, R. M., AND J. V. REMSEN, JR. 1986. Evolutionary processes and patterns of geographic variation in birds, p. 1–69. *In* R. F. Johnston [ed.], Current ornithology. Vol. 4. Plenum Press, New York.