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GENETIC SIMILARITIES BETWEEN SUBSPECIES OF THE WHITE-CROWNED SPARROW[1]

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Abstract. Tissue samples from 121 birds representing two subspecies of the White-crowned Sparrow, *Zonotrichia leucophrys,* and their zone of intergradation, were obtained from a linear series of eight localities along the Pacific coast of California and Oregon. The region sampled included all of the range of *Z. l. nuttalli,* and a portion of the range of *Z. l. pugetensis.* Allozyme variation at 46 presumptive gene loci was examined by means of starch gel electrophoresis; 15 of these loci (32.6%) were polymorphic. Allelic frequencies of these loci are reported here; they are used in an analysis of Wright's *F*-statistics and to estimate gene flow and indices of genetic distance. The population genetic structure of at least one deme within the zone of intergradation differs from that of the two intergrading subspecies, but the subspecies themselves apparently are not genetically differentiated from one another. This conclusion is supported by several lines of evidence: allelic frequencies of the subspecies are not significantly different, as determined by estimates of genetic distance; the association between interlocality F_{st} values and geographic distances is not significant, as measured by the Mantel test; and a reconstruction of the evolutionary relationships based on the genetic distances of Nei, Rogers, and Cavalli-Sforza and Edwards groups localities representing the two subspecies together, but separate from localities within the zone of intergradation. All lines of evidence suggest high levels of gene flow between the subspecies.

Key words: Zonotrichia leucophrys; *subspecies; population genetic structure; electrophoresis; protein polymorphism; gene flow; intergradation.*

INTRODUCTION

Breeding populations of the White-crowned Sparrow, *Zonotrichia leucophrys,* are widely distributed in North America, and among them there is a fair degree of geographic variation. Taxonomic studies have dealt with this variation in different ways, but at present four subspecies are recognized (Banks 1964). These include an eastern Canadian and southern Rocky Mountain race, *Z. l. leucophrys,* an Alaskan and western Canadian race, *Z. l. gambelii,* a Pacific northwest race, *Z. l. pugetensis,* and a central Pacific coast race, *Z. l. nuttalli.* Earlier, Oberholser (1932) recognized the populations in the region of the Warner Mountains of Lake County, Oregon, as a separate subspecies, *Z. l. oriantha,* and subsequently populations in the Sierra Nevada Mountains of California were informally included in *Z. l. oriantha.* To these Miller (1941) formally added the populations of the Rocky Mountains and Great Basin of the United States. All of these populations of *Z. l. oriantha* were subsequently synonymized with *Z. l. leucophrys* by Banks (1964).

Two of the three western subspecies, *Z. l. nuttalli* and *Z. l. pugetensis,* meet and intergrade along the Pacific coast of northern California. The region in which intergradation occurs is a narrow band of coastal chaparral extending from about as far south as Bodega Head in Sonoma County, California, northward through Mendocino County into Humboldt County, California (Grinnell 1928, Blanchard 1941, Banks 1964, Mewaldt et al. 1968, Corbin 1981). This is a region of the coast in which the ecology and floral components change from being predominantly those of a *Baccharis-Lupinus* chaparral south of Cape Mendocino to a coastal evergreen forest on Cape Mendocino (Munz 1959).

Several lines of evidence bear upon the issue of what geographic region constitutes the zone of contact and intergradation between populations currently designated as *Z. l. nuttalli* and *Z. l. pugetensis.* The subspecies *Z. l. nuttalli,* as currently defined, is comprised primarily of nonmigratory birds (Blanchard 1941), whose breeding range is restricted to the coastal areas of central

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TABLE 1. Frequencies of alleles at polymorphic gene loci within demes of *Zonotrichia leucophrys nuttalli, Z. l. pugetensis,* and their intergrades. Demes of *Z. l. nuttalli* include Jalama, Point Piedras Blancas, Marina, and Point Reyes, California. Demes of intergrades include Manchester Beach and Rockport, California. Ferndale, California and Yachats, Oregon, are within the range of *Z. l. pugetensis. n* is the number of genomes examined at each locus and population, *h* is the heterozygosity.

	Demes							
Locus and allele	Jalama	Point Piedras Blancas	Marina	Point Reyes	Man-chester	Rockport	Ferndale	Yachats
Acp a	0.0	0.100	0.0	0.067	0.175	0.063	0.154	0.0
b	0.294	0.500	0.222	0.300	0.650	0.500	0.423	0.643
c	0.412	0.333	0.556	0.567	0.125	0.250	0.269	0.286
d	0.294	0.067	0.222	0.067	0.050	0.188	0.154	0.071
n	34	30	18	30	40	16	26	28
h	0.657	0.624	0.593	0.580	0.529	0.648	0.701	0.500
Gdh-1 a	0.429	0.200	0.300	0.633	1.000	1.000	0.600	0.313
b	0.571	0.800	0.700	0.367	0.000	0.000	0.267	0.469
c	0.0	0.0	0.0	0.0	0.0	0.0	0.133	0.219
n	28	30	20	30	2	10	30	32
h	0.490	0.320	0.420	0.464	0.000	0.000	0.551	0.635
Idh a	0.0	0.0	0.0	0.029	0.025	0.150	0.067	0.0
b	0.111	0.0	0.0	0.059	0.025	0.050	0.033	0.0
c	0.889	1.0	1.0	0.912	0.950	0.800	0.900	1.0
n	36	30	20	34	40	20	30	32
h	0.198	0.000	0.000	0.164	0.096	0.335	0.184	0.000
Me a	0.0	0.0	0.0	0.033	0.0	0.0	0.0	0.0
b	0.111	0.033	0.0	0.200	0.125	0.250	0.200	0.250
c	0.778	0.967	0.900	0.767	0.775	0.700	0.800	0.625
d	0.111	0.0	0.100	0.0	0.100	0.050	0.0	0.125
n	36	30	20	30	40	20	30	32
h	0.370	0.064	0.180	0.371	0.374	0.445	0.320	0.531
Pep-A a	0.038	0.100	0.0	0.167	0.375	0.111	0.100	0.219
b	0.962	0.900	1.0	0.833	0.625	0.889	0.867	0.781
c	0.0	0.0	0.0	0.0	0.0	0.0	0.033	0.0
n	26	30	2	18	40	18	30	32
h	0.074	0.180	0.000	0.278	0.469	0.198	0.238	0.342
Pep-B a	0.0	0.0	0.050	0.176	0.100	0.0	0.100	0.031
b	0.792	0.933	0.950	0.618	0.875	0.833	0.733	0.750
c	0.208	0.067	0.0	0.206	0.025	0.167	0.167	0.219
n	24	30	20	34	40	18	30	32
h	0.330	0.124	0.095	0.545	0.224	0.278	0.424	0.389
Pep-C a	0.0	0.0	0.0	0.038	0.075	0.0	0.0	0.031
b	1.0	0.933	1.0	0.962	0.925	1.0	1.0	0.969
c	0.0	0.067	0.0	0.0	0.0	0.0	0.0	0.0
n	26	30	6	26	40	18	26	32
h	0.0	0.124	0.0	0.074	0.139	0.0	0.0	0.061
6-pgdh a	0.0	0.0	0.0	0.029	0.0	0.0	0.0	0.0
b	0.206	0.100	0.111	0.0	0.0	0.0	0.0	0.067
c	0.324	0.267	0.500	0.676	0.618	0.650	0.500	0.233
d	0.206	0.300	0.167	0.029	0.029	0.050	0.0	0.267
e	0.206	0.233	0.222	0.265	0.206	0.200	0.500	0.167
f	0.059	0.100	0.0	0.0	0.147	0.100	0.0	0.267
n	34	30	18	34	34	20	2	30
h	0.765	0.764	0.660	0.471	0.554	0.525	0.500	0.771
Pgi-1 a	0.0	0.0	0.100	0.0	0.0	0.0	0.0	0.0
b	0.706	0.692	0.600	0.250	0.412	0.600	0.533	0.467
c	0.294	0.308	0.250	0.500	0.353	0.150	0.300	0.367
d	0.0	0.0	0.050	0.194	0.235	0.250	0.100	0.100
e	0.0	0.0	0.0	0.056	0.0	0.0	0.067	0.067
n	34	26	20	36	34	20	30	30
h	0.415	0.426	0.565	0.647	0.651	0.555	0.611	0.633

TABLE 1. Continued.

Locus and allele	Demes							
	Jalama	Point Piedras Blancas	Marina	Point Reyes	Manchester	Rockport	Ferndale	Yachats
Pgm-1 a	0.611	0.808	0.750	0.618	0.625	0.500	0.533	0.656
b	0.389	0.192	0.250	0.382	0.375	0.500	0.467	0.344
n	36	26	20	34	40	20	30	32
h	0.475	0.311	0.375	0.472	0.469	0.500	0.498	0.451
Pgm-2 a	0.882	1.0	0.900	0.750	1.0	1.0	0.967	0.938
b	0.118	0.0	0.100	0.250	0.0	0.0	0.033	0.063
n	34	30	10	8	40	10	30	32
h	0.208	0.0	0.180	0.375	0.0	0.0	0.064	0.117
MP-3 a	1.0	1.0	1.0	0.971	1.0	1.0	1.0	1.0
b	0.0	0.0	0.0	0.029	0.0	0.0	0.0	0.0
n	36	30	20	34	40	20	30	32
h	0.0	0.0	0.0	0.057	0.0	0.0	0.0	0.0
MP-5 a	0.0	0.0	0.0	0.059	0.0	0.0	0.0	0.0
b	0.972	1.000	1.000	0.941	0.700	1.000	1.000	0.938
c	0.028	0.0	0.0	0.0	0.300	0.0	0.0	0.063
n	36	30	20	34	40	20	30	32
h	0.054	0.0	0.0	0.111	0.420	0.0	0.0	0.117
PP-1 a	1.0	0.967	0.900	0.941	1.0	1.0	0.967	1.0
b	0.0	0.033	0.100	0.059	0.0	0.0	0.033	0.0
n	36	30	20	34	40	20	30	32
h	0.0	0.064	0.180	0.111	0.0	0.0	0.064	0.0
PP-3 a	0.0	0.0	0.0	0.000	0.0	0.0	0.167	0.0
b	0.0	0.0	0.0	0.029	0.053	0.0	0.167	0.0
c	1.0	1.0	1.0	0.853	0.737	0.900	0.667	0.0
d	0.0	0.0	0.0	0.059	0.211	0.100	0.0	0.0
e	0.0	0.0	0.0	0.059	0.0	0.0	0.0	0.0
n	2	10	16	34	38	20	24	2
h	0.0	0.0	0.0	0.265	0.410	0.180	0.500	0.0

and northern California (Banks 1964). Clinal shifts in bill length, toe length, and tarsus length occur in northern California in both Mendocino and Humboldt counties, and Banks (1964) considered this to be the primary zone of transition between the subspecies. An analysis of physiological changes associated with molt and migration might be still farther south between Albion, California, in Mendocino County to the south and Capetown, California, in Humboldt County, to the north. Corbin (1981) found that the average heterozygosity of demes was highest in the southern part of Mendocino County, and he suggested that this region was near the center of the zone of intergradation, rather than at the southern edge of the zone proposed by Banks (1964).

The principal objectives of the present study were to determine the degree of genetic differentiation between the two subspecies *Z. l. nuttalli* and *Z. l. pugetensis,* to examine the differences in the genetic structure of these taxa, and to estimate the extent of gene flow between the populations we sampled. A lesser objective was to characterize the zone of transition on the basis of genetic structure.

MATERIALS AND METHODS

A collection of 121 White-crowned Sparrows, *Zonotrichia leucophrys,* was obtained from eight localities along the Pacific coast of California and Oregon during the breeding season of 1978. These localities, shown in Figure 1 and listed in Table 1, were sampled from south to north, beginning on 19 May at Jalama, Santa Barbara County, California, and ending on 27 June at Yachats, Lincoln County, Oregon. Basing the distribution of subspecies on the study of Banks (1964), *Z. l. nuttalli* is represented by specimens from the four southernmost localities (Jalama, Point Piedras

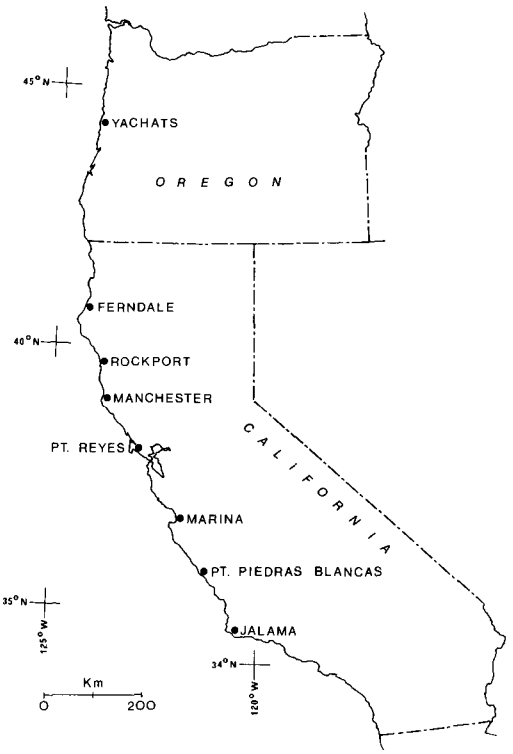

FIGURE 1. Collection localities having breeding individuals of *Z. l. nuttalli* (Jalama, Point Piedras Blancas, Marina, and Point Reyes, California), *Z. l. pugetensis* (Ferndale, California and Yachats, Oregon), and their intergrades (Manchester and Rockport, California).

Blancas, Marina, and Point Reyes, California), *Z. l. pugetensis* by the two northern localities (Ferndale, California and Yachats, Oregon), and specimens from Manchester and Rockport, California, are from the zone of intergradation.

In addition to the preparation of each specimen as a museum skin, the body weight, wing length (unflattened wing chord), tail length, tarsus length, and culmen length of each bird were recorded. These data are available upon request from KWC. For allozyme analyses, samples of blood, liver, pectoral muscle, and heart muscle were taken from each bird at the time of collection. Except for the blood samples, these tissues were immediately frozen in liquid nitrogen. Blood samples were taken in heparinized syringes, and after separation of plasma and red cells by settling overnight at 4°C, these tissues also were stored frozen in liquid nitrogen. The museum skins have been deposited with the Bell Museum of Natural History, University of Minnesota, Minneapolis, and the Museum of Vertebrate Zoology, University of California, Berkeley. Frozen tissues continue to be stored in liquid nitrogen at the University of Minnesota.

Prior to electrophoresis, extracts of liver and muscle tissues were prepared in a solution of phenoxyethanol, sucrose, and phosphate buffer as described by Nakanishi et al. (1969). Both tissue extracts and blood plasma samples were centrifuged for 30 min at 20,000 rpm at 4°C to remove cellular debris. Samples were analyzed for allozyme content and variation by means of horizontal starch gel electrophoresis following the procedures of Corbin et al. (1974), Corbin (1977), and Barrowclough and Corbin (1978).

Forty-six presumptive gene loci were analyzed for allozyme polymorphisms. These loci and their most recent Enzyme Commission numbers are as follows (abbreviations of the polymorphic loci are given in parentheses): adenosine deaminase (3.5.4.4), alkaline phosphatase (3.1.3.1), acid phosphatase (Acp, 3.1.3.2), three creatine kinases (2.7.3.2), two glutamate dehydrogenases (Gdh-1 and Gdh-2, 1.4.1.3), two aspartate aminotransferases (2.6.1.1), α-glycerol phosphate dehydrogenase (1.1.1.8), α- and β-hemogoblin, two NADP-dependent isocitrate dehydrogenases (Idh-1 and Idh-2, 1.1.1.42), lactate dehydrogenase muscle and heart forms (1.1.1.27), malic enzyme (Me, 1.1.1.40), two malate dehydrogenases (1.1.1.37), three mannose phosphate isomerases (5.3.1.8), valyl-leucine dipeptidase (Pep-A, 3.4.11.11), leucyl-glycyl-glycine dipeptidase (Pep-B, 3.4.11.4), leucyl-alanine dipeptidase (Pep-C, 3.4.11.), 6-phosphogluconate dehydrogenase (6-Pgdh, 1.1.1.44), two glucose-6-phosphate isomerases (Gpi-1 and Gpi-2, 5.3.1.9), three glucose phosphomutases (Pgm-1 through Pgm-3, 5.4.2.2), two L-iditol dehydrogenases (1.1.1.14), two superoxide dismutases (1.15.1.1), seven nonspecific muscle proteins (MP-1 through MP-7), and three nonspecific plasma proteins (PP-1 through PP-3).

The localities sampled are treated here as separate demes because even the shortest geographic distance between collection sites (89 km) is more than 200 times greater than the average lifetime dispersal distance for *Z. l. nuttalli*, which is approximately 600 m (Baker and Mewaldt 1978). The microcomputer program GENESYS, writ-

ten in Pascal by K.W.C., was used to calculate allelic frequencies, heterozygosities, genetic distances, F_{st} values, and a Wagner tree with fitted branch lengths. Another Pascal program written by K.W.C. was used to construct a consensus Wagner tree based on the bootstrap method suggested by Felsenstein (1985). Genetic distances between demes were estimated by the methods of Cavalli-Sforza and Edwards (1967), Nei (1972, 1978), and Rogers (1972). F_{st} values were estimated by the method of Wright (1965, 1978). Both Nei's D and Wright's F_{st} values are corrected for sampling error due to sample sizes. Chi-squared values testing the deviation of F_{st} from zero are estimated as $2nF_{st}$ using both the original data set and a pooled data set. In the latter case, the data of Table 1 were combined under only two alleles at each locus, the observed value of the most frequent allele at a locus, and 1.0 minus that frequency.

The construction of the dendrogram depicting the genetic relationships of the localities is based on the minimum spanning network algorithms of Farris (1972, 1973), and the consensus Wagner network was produced as the consensus of 100 trees generated from randomly selected subsets of the allelic frequencies in Table 1, but bootstrapped over loci (Felsenstein 1985). Each of the 100 trees was based, therefore, on genetic distances estimated from a random sample of 15 polymorphic loci and the 31 monomorphic loci. Nodes and branch assignments were established by majority rule. Thus, the nodes chosen occurred either in a majority of the 100 trees or were the most frequent alternatives compatible with the majority-rule branch and node assignments.

Gene flow between localities has been estimated (1) by examining the relationship between the conditional average frequency, $\bar{p}(i)$, of Slatkin (1981) and the proportion of localities having given values of $\bar{p}(i)$, and (2) with Nm (Slatkin 1985) which is based on the linear relationship between gene flow and the conditional average frequencies of alleles found in only one deme. The former method provides a qualitative measure of gene flow whereas the latter estimates the number of immigrants per generation. In estimating gene flow using the equation $\ln[p(1)] = a \ln(Nm) + b$ (Slatkin 1985), where $a = -0.505$ and $b = -2.44$, estimates of Nm have been adjusted for sampling error by dividing the initial values of Nm by a factor of 0.605 ($n/25$), as suggested by Slatkin (1985), where n for our study = 15.13 individuals.

RESULTS

VARIABILITY AND GENE FLOW

Fifteen of the 46 loci examined, or 32.6%, were polymorphic. The allelic frequencies of these polymorphic loci are presented in Table 1. As reported previously (Corbin 1981), the average observed heterozygosity, $\bar{H}$, at the eight localities ranged from 0.043 to 0.078, with a mean of 0.056 $\pm$ 0.011. For comparison to these observed values of heterozygosity we present here the calculated heterozygosities for individual loci, estimated as $1 - \Sigma x_i^2$, where x_i is the ith allele of locus x. These locus by locus values are given in Table 1, and when averaged over all loci, including the 31 that are monomorphic, yield an overall mean calculated value of H equal to 0.0994 $\pm$ 0.0290 ($\bar{x} \pm$ SE) for these eight localities of *Z. leucophrys*.

Alleles of several loci appear to be fixed in one or more localities (Table 1). In a few cases, a rare allele is found in only one or a few localities, but in no case is an allele that is fixed in one locality not found in any other locality. The distribution of these rare alleles has been used to estimate gene flow involving the four populations that possessed private alleles (Slatkin 1985). Point Piedras Blancas has one private allele at the Pep-C locus giving values of $\bar{p}(i) = 0.067$ and $Nm = 2.783$. Marina has one private allele at the Pgi-1 locus with $\bar{p}(i) = 0.100$ and $Nm = 1.259$. Point Reyes has private alleles at Me, 6-pgdh, MP-3, MP-5, and PP-3, with $\bar{p}(i) = 0.042$ and $Nm = 7.016$. Ferndale has private alleles at Pep-A and PP-3, with $\bar{p}(i) = 0.100$ and $Nm = 1.259$.

The above confirms the qualitative estimate of gene flow based on the earlier method of Slatkin (1981) which utilizes the pattern of the relationship between $\bar{p}(i)$ and i/d, where i/d is the ratio of the incidence of alleles to the number of localities sampled. This pattern, shown in Figure 2, indicates high levels of gene flow among localities.

PHYLOGENETIC RELATIONSHIPS

Tables 2 and 3 present the genetic distances between localities. Nei's (1978) unbiased genetic distances, D, and Rogers' (1972) distances are

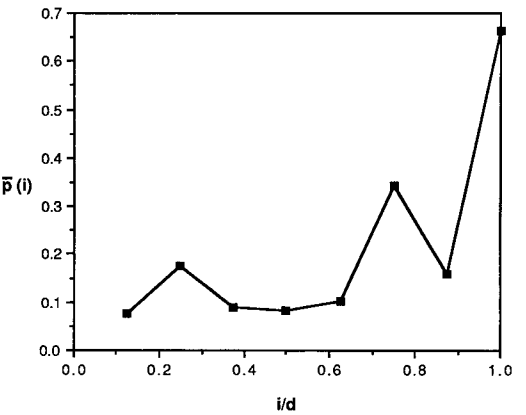

FIGURE 2. Relationship between the conditional average frequency, $\bar{p}(i)$, of Slatkin (1981) and the ratio of the incidence of alleles within demes, i, to the total number of demes sampled, d. Based on the theoretical analyses of Slatkin (1981) this pattern indicates relatively high levels of gene flow among demes.

given in Table 2 for comparison to other studies of this type. Wright (1978), however, found these measures to be less satisfactory than those of Cavalli-Sforza and Edwards (1967), and their arc and chord distances are given in Table 3. Following the lead of Wright (1978), we have not included the factor of $(2\sqrt{2})/\pi$ in the calculation of the chord distances since, for this metric, this would mean the maximum distance between taxa could be only 0.9003 rather than 1.0.

To approximate the phylogenetic relationships of the demes sampled here, the distance metrics of Tables 2 and 3 were used to generate Wagner trees (Farris 1972, 1973). The topologies of these three trees were essentially identical, but the best fit between observed and fitted distances is obtained for arc distances. We then prepare the consensus tree as the composite of 100 analyses, with the following results: The first taxa joined are Marina and Point Piedras Blancas which join their connecting node (9) in 55% and 50% of the trees, respectively. Jalama next joins the tree initially at node 9 and ultimately via node 10 in 45% of all trees vs. Ferndale at 9%. Yachats next joins the tree initially at node 10 and ultimately via node 11 in 37% of all trees, vs. Point Reyes at 24%. The next step is ambiguous with Point Reyes and Ferndale being equally likely options initially connecting at node 11, but ultimately via interconnecting nodes 12 and 13 in 28% and 29% of all trees, respectively. The last two taxa, Manchester and Rockport, ultimately connect first via node 14 and then node 12 in 62% and 49% of all trees.

The consensus tree is shown in Figure 3 with the distances between taxa and nodes being the fitted arc distances of Cavalli-Sforza and Edwards. We have arbitrarily rooted this tree midway between the most distantly related demes (Point Piedras Blancas and Point Reyes). Statistics that measure the goodness-of-fit between the values used to construct this dendrogram and the original arc distances in Table 3 are as follows: total homoplasy = 0.2048, the cophenetic correlation coefficient = 0.8871, the percent standard deviation of Fitch and Margoliash (1967) = 13.60, and the F value of Prager and Wilson (1976) = 10.72.

GENETIC STRUCTURE

Wright's (1978) F_{st} values for all possible interlocality, paired comparisons are given below the diagonal in Table 4. These estimates incorporate the variation at all polymorphic loci for each of the respective comparisons. Chi-squared values for the deviation of the F_{st} values from 0.0 for the unpooled data are given above the diagonal in Table 4. The overall mean F_{st} value for all

TABLE 2. Genetic distance estimates for comparisons between demes of *Zonotrichia leucophrys*. Nei's (1978) unbiased genetic distances, D, are given above the diagonal, and Rogers' (1972) distances are given below the diagonal.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Jalama (1)	—	0.0029	0.0000	0.0063	0.0195	0.0099	−0.0006	0.0034
Point Piedras Blancas (2)	0.0329	—	0.0009	0.0138	0.0247	0.0206	0.0052	0.0044
Marina (3)	0.0273	0.0269	—	0.0067	0.0236	0.0155	0.0022	0.0072
Point Reyes (4)	0.0434	0.0592	0.0493	—	0.0109	0.0069	−0.0031	0.0077
Manchester (5)	0.0614	0.0646	0.0676	0.0526	—	0.0039	0.0027	0.0142
Rockport (6)	0.0437	0.0544	0.0557	0.0468	0.0371	—	−0.0030	0.0116
Ferndale (7)	0.0390	0.0476	0.0485	0.0373	0.0485	0.0328	—	−0.0006
Yachats (8)	0.0332	0.0368	0.0445	0.0460	0.0511	0.0471	0.0415	—

TABLE 3. Genetic distance estimates for comparisons between demes of *Zonotrichia leucophrys*. Cavalli-Sforza and Edwards' (1967) arc distances are given above the diagonal, and their chord distances are given below the diagonal. Following the practice of Wright (1978), chord distances have not been multiplied by a factor equal to $(2\sqrt{2})/\pi$.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Jalama (1)	—	0.0438	0.0422	0.0645	0.0750	0.0564	0.0625	0.0426
Point Piedras Blancas (2)	0.0484	—	0.0446	0.0720	0.0780	0.0629	0.0626	0.0509
Marina (3)	0.0467	0.0493	—	0.0692	0.0878	0.0747	0.0674	0.0608
Point Reyes (4)	0.0710	0.0792	0.0763	—	0.0683	0.0653	0.0508	0.0627
Manchester (5)	0.0822	0.0852	0.0960	0.0752	—	0.0427	0.0670	0.0619
Rockport (6)	0.0619	0.0687	0.0819	0.0720	0.0471	—	0.0506	0.0602
Ferndale (7)	0.0687	0.0688	0.0741	0.0561	0.0737	0.0556	—	0.0581
Yachats (8)	0.0471	0.0562	0.0670	0.0690	0.0678	0.0659	0.0638	—

demes, loci, and alleles is 0.0562, which is significantly different from zero ($\chi^2 = 169.27$, $P < 0.01$, df = 105). For the pooled data set having only two alleles at each locus, $\chi^2 = 189.38$, df = 105, and $P \ll 0.001$.

Figure 4 presents information on the relationship between interlocality F_{st} values, given in Table 4, and the distance in kilometers between localities. These distances are measured along the dispersal routes of the coastal chaparral rather than from point to point on a map. The solid squares in Figure 4 indicate intersubspecific comparisons, the solid circles are the intrasubspecific comparisons, and the open circles show comparisons involving one or the other of the two localities within the zone of contact to each of the other six localities.

Because the matrix values of interlocality F_{st} estimates are based on dependent subsets of the original allelic frequency data, an appropriate test of the relationship between F_{st} and interlocality distance is the Mantel test (Mantel 1967, Schnell et al. 1985). For the full data set, the association between F_{st} and distance yields a Z value of 402.16 which is not significantly different from that expected (381.53) for the random association of the elements in the two matrices ($t = 0.6593$, $P > 0.05$). For a restricted data set involving only the interlocality F_{st} values and geographic distances between demes within the range of *Z. l. nuttalli*, $Z = 47.82$, which again is not significantly different from the expected value, 43.95, assuming random association of elements ($t = 0.6751$, $P = 0.05$). Having only two localities represented, a comparable Mantel test cannot be carried out for *Z. l. pugetensis*.

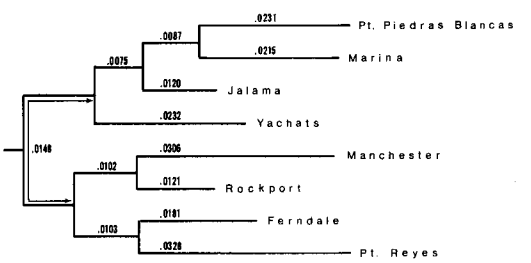

FIGURE 3. Dendrogram showing the relationships of demes of *Zonotrichia leucophrys* based on the consensus of 100 maximum parsimony networks constructed using the algorithms of Farris (1972, 1973). Branch lengths are the fitted values in units of Cavalli-Sforza and Edwards' (1967) arc distance. The tree is rooted arbitrarily midway between the greatest fitted distance between demes, i.e., between Point Piedras Blancas and Point Reyes, California. See text for a discussion of the branch and node assignment percentages in the consensus tree.

DISCUSSION

F_{st} AND POPULATION GENETIC STRUCTURE

When external morphological variation is sufficient to recognize subspecies one expects a priori to find evidence for genetic structure at the level of protein variation. Genetic structure, however, might not be entirely concordant with morphological variation. As in the case of morphological variation, genetic structuring would be due to variation in the effects of natural selection, in the extent of gene flow between subdivisions, or in the degree of inbreeding and genetic drift. Conversely, the absence of genetic structure indicates that the action of these processes has not resulted in significant genetic differentiation within the taxon. In this study we have a case of physiological and external morphological variation with the apparent absence of significant genetic structuring of the subspecies.

Genetic structure is revealed as patterned vari-

TABLE 4. Values of F_{st}, corrected for sample variance (Wright 1978), and their associated χ^2 values for pairwise comparisons between demes of *Zonotrichia leucophrys*. Each F_{st} value is the mean of values obtained for the 15 polymorphic loci listed in Table 1. These are given below the diagonal, and χ^2 values are above the diagonal. Each of the latter have 1 df and none is significant at the 0.05 level.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Jalama (1)	—	0.625	0.287	0.838	2.488	0.293	0.490	0.602
Point Piedras Blancas (2)	0.0103	—	0.156	1.581	1.500	0.793	0.968	0.570
Marina (3)	0.0057	0.0034	—	0.844	1.915	0.350	0.763	1.047
Point Reyes (4)	0.0134	0.0272	0.0177	—	1.031	0.446	0.217	0.821
Manchester (5)	0.0360	0.0232	0.0353	0.0155	—	0.338	0.459	0.878
Rockport (6)	0.0058	0.0172	0.0098	0.0093	0.0062	—	0.140	0.375
Ferndale (7)	0.0082	0.0175	0.0170	0.0038	0.0072	0.0031	—	0.504
Yachats (8)	0.0097	0.0099	0.0222	0.0138	0.0133	0.0079	0.0089	—

ation in the distribution of genotypes and allelic frequencies, and can be examined and quantified by means of *F*-statistic analysis (Wright 1978). Low values of F_{st} are associated with panmixia, whereas increasingly larger values result from reduced levels of gene flow. If, in an analysis of the genetic structure of a species, one finds that F_{st} values are not significantly different from zero, then subdivision of the taxon probably does not account for whatever genetic variation may exist.

If the number of alleles at polymorphic loci is only two and if the number of genomes assayed at each locus of all localities is identical then the testing of the deviation of F_{st} from zero is straightforward. Under these conditions $2nF_{st}$ is distributed approximately according to χ^2 (Cockerham 1973), where $2n$ is the total number of genomes sampled over all localities. For several reasons these conditions are seldom met in studies of natural populations. When sampling variation exists it seems obvious that estimates of F_{st} should be weighted according to the number of individuals sampled from each locality, but the use of Cockerham's (1973) method does not correct for this kind of sampling error. In an earlier F_{st} analysis of our allelic frequency data (Corbin 1981) the method of Cockerham was used and the earlier results are significantly different from those presented here. It is perhaps important to note here that Cockerham's F_{st} values are identical to the uncorrected or biased values of Wright (1978).

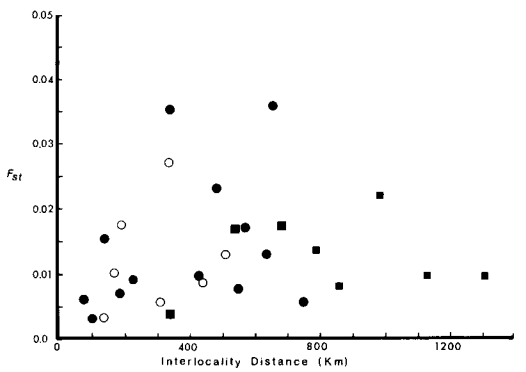

FIGURE 4. Association between interlocality dispersal distances and F_{st} values for paired comparisons of the collecting localities shown in Figure 1. Comparisons between demes of different subspecies, e.g., Jalama (*Z. l. nuttalli*) vs. Yachats (*Z. l. pugetensis*), are indicated with solid squares. Solid circles designate intrasubspecific comparisons, and comparisons involving the two localities (Manchester and Rockport) within the zone of intergradation between subspecies are indicated with open circles. Since both measures consist of dependent data sets, the Mantel test is used to measure the degree of association between them as discussed in the text.

Neither method makes an allowance for having more than two alleles at a locus, but we have approached this problem as discussed above in the sections on methods and results. On the basis of the differences in the two estimates of χ^2 it appears that in spite of whatever theoretical problems there may be with variation in the number of genomes per deme and the number of alleles per locus, the error associated with estimating χ^2 as $2nF_{st}$ may actually result in a more conservative test of the deviation of F_{st} values from zero if an unmodified data set is used.

The significant χ^2 values, associated with their respective F_{st} values, seem to indicate that some genetic subdivision of *Z. leucophrys* does exist. A priori, one would expect to find that such subdivision coincides with the delineation of subspecies, but this is contradicted by the results of the Mantel test. Recall that there was no association between interlocality F_{st} values and interlocality geographic distances, which indicates

that the eight demes examined here are part of a single population. An alternative possibility is suggested by the fact that the allelic frequency and heterozygosity data of Manchester, California, are considerably different from those of the other localities.

To test the possibility that this deme within the zone of intergradation may be genetically unlike those of the intergrading subspecies, we repeated the calculations of F_{st} and χ^2 on a data set modified by excluding the allelic frequency data for Manchester. When this was done, the value of F_{st} was no longer significantly different from zero ($F_{st} = 0.0360$, $\chi^2 = 91.21$, df = 90, $P > 0.05$).

There appears to be an explanation, therefore, for the contrasting results of the various kinds of analyses involving F_{st} values. Not only is the overall F_{st} value not significantly different from zero when the data for Manchester are excluded, but also the paired F_{st} data graphed in Figure 4 show little or no association with geographic distance between localities. The latter is confirmed by the results of the Mantel test and is consistent with the general pattern of high levels of gene flow among demes (Fig. 2 and *Nm* greater than 1.0). These lines of evidence indicate, therefore, that the genetic structures of the intergrading subspecies are similar, that there is little genetic differentiation between the subspecies in spite of the morphological differences that exist, and that at least one deme within the zone of intergradation has diverged in genetic structure from those of the intergrading subspecies.

On the basis of the above analyses we conclude that *Z. l. nuttalli* and *Z. l. pugetensis* constitute a single breeding population. Others (e.g., Baker 1974, 1975; Baker and Mewaldt 1978) have argued that the dialects of *Z. leucophrys,* which are especially complex and diverse in *Z. l. nuttalli* (Baptista 1975), may reduce gene flow within the subspecies if individuals of one dialect are most likely to mate within the region of their own dialect. We, on the other hand, find evidence for high levels of gene flow among all localities, with the possible exception of Manchester. Conversely, if gene flow were restricted as proposed by Baker and Mewaldt the F_{st} values should be significantly greater than zero when localities are compared over the entire range of the subspecies, and would contribute to an increased correspondence between F_{st} and distance. We find no evidence for these effects and conclude that gene flow throughout the range of *Z. l. nuttalli* and *Z. l. pugetensis* is not significantly impeded by differences in dialects.

This leaves unanswered the question of why the genetic structure and composition of a locality within the zone of intergradation between genetically undifferentiated subspecies should be unique in comparison to the parental subspecies. Clues to a plausible explanation for this anomaly lie in the patchy nature of the species' distribution within the zone of contact and the sizes of demes in that region. Based on superficial field observations, it is our impression that these demes are relatively small, some having fewer than 10 birds, and they are isolated from one another. These are conditions that would favor genetic drift in demes within the contact zone, and genetic drift could account for the marked differences in allelic frequencies observed in the zone of intergradation (see Table 1).

ZONE OF INTERGRADATION AND TAXONOMY

The high degree of genetic similarity among localities of *Z. l. nuttalli* and *Z. l. pugetensis* is demonstrated in the genetic distance matrices of Tables 2 and 3. Opposite to that expected for subspecies that are genetically distinct, values for comparisons between localities of different subspecies all fall within the range of values obtained for intrasubspecific comparisons (Nei's distance: intrasubspecific range = −0.0006 to 0.0130, intersubspecific range = −0.0006 to 0.0077; Rogers' distance: intrasubspecific range = 0.0273 to 0.0592, intersubspecific range = 0.0332 to 0.0485; Cavalli-Sforza and Edwards' arc distance: intrasubspecific range = 0.0422 to 0.0720, intersubspecific range = 0.0426 to 0.0674; Cavalli-Sforza and Edwards' chord distance: intrasubspecific range 0.0467 to 0.0792, intersubspecific range = 0.0471 to 0.0741).

On the other hand, the largest genetic distances involve paired comparisons between Manchester, California and some other locality. These similarities and differences are summarized and emphasized in the dendrogram of Figure 3, in which at least one deme of each subspecies segregates with demes of the other subspecies. The two localities sampled from the zone of contact (Manchester and Rockport) are more closely allied to one another than to localities of either intergrading subspecies. We note, however, that a few of the fitted distances of the dendrogram

deviate considerably from observed values, and much of the total homoplasy is due to the position of Point Reyes in the Wagner tree.

We interpret these results to mean that in spite of some morphological differentiation between the currently recognized subspecies, *Z. l. nuttalli* and *Z. l. pugetensis,* other portions of their genomes have not diverged significantly. Thus, a broad underlying genetic basis for the recognition of these two subspecies apparently does not exist.

Finally, we turn to the taxonomic implications of our study. If our data are representative of the degree of genetic differentiation throughout the genomes of *Z. l. nuttalli* and *Z. l. pugetensis,* then it follows that the continued recognition of these taxa as subspecies will be based on a rather restricted set of characters. This, of course, would emphasize the importance of the morphological variation used by Banks (1964), who delineated these subspecies on the basis of clinal shifts of five morphological characters, i.e., adult body weight, and the lengths of the wing, bill, tarsus, and middle toe. However, the clinal shifts involving these characters do not all occur in the same geographic region of Cape Mendocino, and none of the shifts coincides with the primary transition zones observed in other suites of physiological and biochemical characters studied respectively by Mewaldt et al. (1968) and Corbin (1981), and in this study. Indeed, as seen in Table 1, discordance in allelic frequency patterns within the zone of intergradation is a striking feature of these data. In the zone of intergradation there are abrupt shifts in allelic frequency at every polymorphic locus, and these changes occur in several different regions of the contact zone.

Banks (1964) placed the division between *Z. l. nuttalli* and *Z. l. pugetensis* in the vicinity of Cape Mendocino, presumably near the border between the counties of Humboldt and Mendocino. Based on the studies of Mewaldt et al. (1968) and Corbin (1981) the division line between these populations lies to the south of Manchester, California near the border between Mendocino County and Sonoma County, California. Based on the allelic frequency data presented here, and depending upon which locus one examines, the transition occurs anywhere from as far south as Point Reyes in Marin County, California, to as far north as Rockport in the northern part of Mendocino County, California, but south of Cape Mendocino itself. Thus, there is an obvious lack of concordance among the characters used to define the transition from *Z. l. nuttalli* to *Z. l. pugetensis.* In the case of the allelic frequency data, however, one sees a series of disruptions in rather shallow north-south clines, with the allelic frequencies differing significantly only within the zone of transition between the northern and southern parts of the distribution.

Considering these data in light of the problems systematists have long experienced in finding concordant clines of morphological characters within zones of intergradation leads us to the following hypothesis. Given that (1) various suites of characters, whether they be those of gross morphology, physiology, or genetics, exhibit discordant patterns of variation within zones of intergradation, and (2) that optimum habitat within such zones may be patchy and populations are both smaller in size and more isolated, then the common cause of this variation may be genetic drift. If this were so then no single trait could or should be used to delineate subspecies. Rather, one would expect the parental subspecies to be separated by a zone composed of intergrades of various phenotypic and genotypic compositions.

The degree to which intergrades exhibit such variation will depend upon the extent of genetic differentiation between the parental subspecies. For *Z. l. nuttalli* and *Z. l. pugetensis* we conclude they are not well differentiated genetically, and that gene flow between them is sufficient to swamp out differences among their separate populations. However, within the zone of contact and intergradation between these subspecies there exists a region near the middle of the zone of intergradation in the vicinity of Manchester, California, that has a unique genetic structure in comparison to that of the two intergrading subspecies.

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