GENIC HETEROZYGOSITY IN THE WHITE-CROWNED SPARROW: A POTENTIAL INDEX TO BOUNDARIES BETWEEN SUBSPECIES

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ABSTRACT.—Estimates of the average heterozygosity, $\bar{H}$, based on the genic variability at 44 loci, are presented for eight Pacific coastal populations of the White-crowned Sparrow (*Zonotrichia leucophrys*). Four of the localities sampled span the geographic distribution of *Z. l. nuttalli*, at least one represents *Z. l. pugetensis*, and the others fall along a north-south transect of the zone of intergradation between these two subspecies. Estimates of $\bar{H}$ are highest in the zone of intergradation and decrease significantly with distance north or south from the presumptive midpoint of the contact zone. This decrease closely approximates an exponential decay curve that peaks in the contact zone, decreases exponentially both northward and southward, and asymptotically approaches a value equal to the average heterozygosity of passerine birds. *Received 15 September 1980, accepted 7 April 1981.*

DURING the 1940's and early 1950's, the concept of the subspecies became a major focus for taxonomists who sought to reconcile intraspecific geographic variation, which is often manifested as discordant clines, with the imperfect constraints of the nomenclatural system (Wilson and Brown 1953, Bogert 1954, Burt 1954, Clench 1954, Edwards 1954, Hubbel 1954, Sibley 1954). It was generally recognized that subspecies and clines are unique concepts, the former being taxonomic while the latter are evolutionary in nature.

Mayr (1942) had defined the subspecies, or geographic race, as "a geographically localized subdivision of the species, which differs genetically and taxonomically from other subdivisions of the species." He subsequently took the position that the recognition of subspecies, as a taxonomic concept, held little meaning other than to provide the taxonomist a means with which to designate phenotypically similar populations (Mayr 1963). Indeed, Wilson and Brown (1953) argued against the formal recognition of subspecies because of the misuse of the trinomial, and by 1954 taxonomists of several fields were reaching a concensus: the trinomial had been misapplied in some groups and the designation of subspecies required both extensive knowledge about a species and competent judgment on the part of the taxonomist (Bogert 1954, Burt 1954, Clench 1954, Edwards 1954, Hubbel 1954, Sibley 1954).

In practice, subspecies have been recognized in some groups, such as Aves, when 75% or more of the individuals under consideration can be assigned to one or another phenotype based upon morphological variation (Mayr 1942). Physiological, biochemical, and ethological variation rarely have been used as criteria for the recognition of subspecies. Unfortunately, however, carefully defined criteria for describing subspecies often have not been applied, with the result being the recognition of subspecies based on seemingly trivial and nonsignificant differences.

Apart from the problem of assigning an individual to a given subspecies, the delineation of the subspecific boundaries *per se* may be even more arbitrary and dependent upon the judgment of the taxonomist, without regard to fundamental biological premises. Unlike species, subspecies cannot be delimited on the basis of discontinuities either in time or space as a result of the cessation of gene flow, except

when populations are geographically isolated. Among subspecies, the genes possessed by one deme necessarily pass, via juvenile and adult dispersal, into other demes both within and between subspecies. But as a result of both differertial selection at a locus and differing selective regimes among loci, gene flow varies among loci, causing the suites of morphological, physiological, biochemical, and ethological traits to vary independently from one subspecies to another. The result of this is that clines across zones of primary and secondary intergradation often are not concordant, and what appears to be the midpoint between subspecies for one character gradient may not coincide in space with that of another cline. Authors have not agreed upon how such situations should be dealt with taxonomically. Some would recognize several subspecies delineated by breaks in one or more of the clines (Hebard 1936). Others would choose to recognize the existence of discordant clines simply as the variation of independent characters within a single subspecies (Wilson and Brown 1953, Burt 1954).

Of those factors that lead to differentiation of subspecies versus species (differential mutation, selection, drift, and gene flow), only gene flow is likely to play a significantly different role. Therefore, for well-differentiated subspecies it is plausible to expect parameters that measure genetic divergence, such as Nei's (1972) and Rogers' (1972) indices of genetic distance or Wrightian F-statistics (Wright 1951; Cockerham 1969, 1973), to vary in predictable ways that correlate with the distribution of subspecies boundaries. These parameters, however, embody many of the shortcomings of the characters themselves when it comes to delineating the boundaries. They are powerful measures of homogeneous subsets of populations, but they cannot distinguish boundaries unless gaps exist, and their rigorous application is highly dependent upon careful and extensive sampling procedures. But gaps in character distributions do not exist *per se* between subspecies, unless the subspecies are geographically isolated.

There is yet another measure that has not been used previously to measure genetic, or subspecific, boundaries, and its application appears to depend on gene flow rather than its restriction or cessation. This measure is that of genic heterozygosity. In this paper I show that mean genic heterozygosity, $\bar{H}$, decreases significantly as a function of the distance from the zone of intergradation between two subspecies of the White-crowned Sparrow (*Zonotrichia leucophrys*). Theoretical arguments for this relationship are summarized briefly. The data presented here were obtained from birds collected along a transect through the zone of intergradation of the Pacific coast subspecies, *Zonotrichia leucophrys nuttalli* and *Z. l. pugetensis*. Morphologically, these two subspecies are poorly differentiated (Banks 1964, Mewaldt et al. 1968), but I summarize here and detail elsewhere that they have diverged genetically to the point that they are significantly different as measured by Wrightian F-statistic analyses.

MATERIALS AND METHODS

In this study, heterozygosity of an individual or a local population is based on the genotypes of loci that code for enzymes and structural proteins; 44 loci are included in this analysis. Proteins coded for by these loci were obtained from red blood cells, blood plasma, breast muscle, and liver tissue of 125 individuals of *Zonotrichia leucophrys*. Eight localities in California and Oregon were sampled in May, June, and July 1978 (Table 1 and Fig. 1). All tissue samples were placed in liquid nitrogen (−182°C) immediately after collection. Blood samples were obtained by cardiac puncture and, after separation of plasma and red cells by settling overnight at 4°C, these samples also were placed in liquid nitrogen.

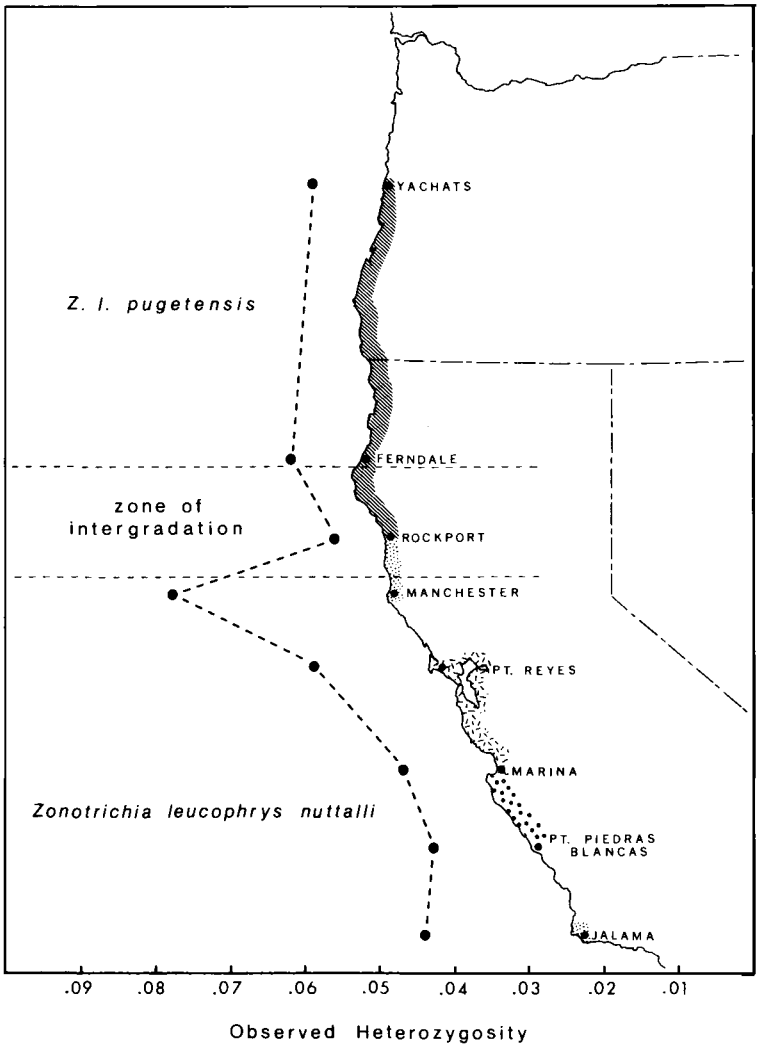

Fig. 1. Localities sampled in California and Oregon that constitute homogeneous subsets are connected by similar stippling when F_{st} values based on allelic and genotypic distributions are not significantly different from zero. Discontinuities, between Manchester and Pt. Reyes for example, indicate that F_{st} values calculated for the combination of these two localities are significantly greater than zero. The solid circles connected by the dashed line are values of average observed heterozygosity, $\bar{H}$, from Table 1. The limits to the zone of intergradation are those proposed by Mewaldt et al. (1968).

Extracts of tissues were prepared according to the procedure of Corbin et al. (1974) and Barrowclough and Corbin (1978). Extracts were analyzed by horizontal starch gel electrophoresis and allozymes were located by using the procedures of Corbin et al. (1974) and Barrowclough and Corbin (1978), which were optimized for avian material.

These electrophoretic analyses provide information about the genotypic distributions at 44 genetic loci, 15 of which were polymorphic (Corbin, in prep.). The distributions of those genotypes involving the most frequent allele at each of 12 of these polymorphic loci were used to calculate F_{st} values (Wright 1951; Cockerham 1969, 1973) for all possible sets of adjacent populations taken in pairs and in combination with adjacent sets along the Pacific coast. Populations within sets having F_{st} values that are not signif-

TABLE 1. Observed individual heterozygoisty (h), average heterozygosity ($\bar{H}$), and standard error (SE) for populations of White-crowned Sparrows, expressed as the ratio of heterozygous loci/total number of loci scored per individual.

Sample locality	N	Range of h	$\bar{H}$	SE
Jalama, California	18	0.000(0/44)–0.122(5/41)	0.044	0.007
Pt. Piedras Blancas, California	15	0.000(0/44)–0.091(4/44)	0.043	0.007
Marina, California	10	0.000(0/42)–0.095(4/42)	0.047	0.011
Pt. Reyes, California	21	0.023(1/43)–0.146(6/41)	0.057	0.007
Manchester, California	20	0.023(1/44)–0.140(6/43)	0.078	0.008
Rockport, California	10	0.024(1/41)–0.091(4/44)	0.056	0.006
Ferndale, California	15	0.024(1/42)–0.093(4/43)	0.062	0.005
Yachats, Oregon	16	0.000(0/44)–0.136(6/44)	0.059	0.010

icantly different from zero, and which therefore have nonsignificant χ^2 values, form homogeneous subsets within a subspecies with respect to allelic and genotypic distributions.

RESULTS

The results of the F_{st} analyses are summarized in Fig. 1 by linking localities within homogeneous sets by means of specific types of stippling. Thus, the population of Jalama, California is distinct and constitutes an isolated subset. Populations of Pt. Piedras Blancas, Marina, and Point Reyes, California form two homogeneous subsets interconnected by the population held in common at Marina. Populations at Manchester, Rockport, and Ferndale, California and Yachats, Oregon form another two homogeneous subsets interconnected by the population shared at Rockport. A statistically significant discontinuity in allelic and genotypic frequencies exists between Pt. Reyes and Manchester, California ($F_{st} = .6278$, $\chi^2_{12} = 39.56$, $P < 0.005$). Likewise the distributions at Jalama are significantly different from those of more northern populations ($F_{st} = 0.3980$, $\chi^2_{12} = 24.88$, $P < 0.025$). These discontinuities are indicated in Fig. 1 by the absence of stippling.

The genotypic data are used here primarily to estimate individual, *observed* heterozygosity, h (Table 1), which is calculated as the total number of heterozygous loci possessed by an individual divided by the total number of loci assayed for that individual. The maximum number of loci scored for any individual was 44 and the minimum was 39. These values of h were averaged over individuals to obtain the average *observed* heterozygosity, $\bar{H}$, of a locality along with the associated variance of that parameter (Table 1 and Fig. 1). Thus, even a population sample of only 10 individuals, such as for Marina, California, provides between 390 and 440 data points upon which the value of $\bar{H}$ for that population is based, with each individual providing independent estimates of h. An alternative method for estimating $\bar{H}$ is based upon the allelic frequencies at each locus assayed for a given population. In this case, h is estimated for loci, not individuals, and equals $1 - \Sigma x_i^2$, where x_i is the ith allele at locus x, and $\bar{H}$ is the average *calculated* heterozygosity, obtained by averaging h over loci. For reasons discussed below, this latter method of estimating $\bar{H}$ is not used here.

Figure 1 shows observed $\bar{H}$ for each locality plotted by locality from the southernmost limit of the distribution of *Zonotrichia leucophrys nuttalli* at Jalama, California northward through the zone of intergradation with *Z. l. pugetensis* to Yachats, Oregon. Figure 2 presents two linear regressions of observed $\bar{H}$ on the distance in kilometers from Manchester, California, a locality that falls close to the presently

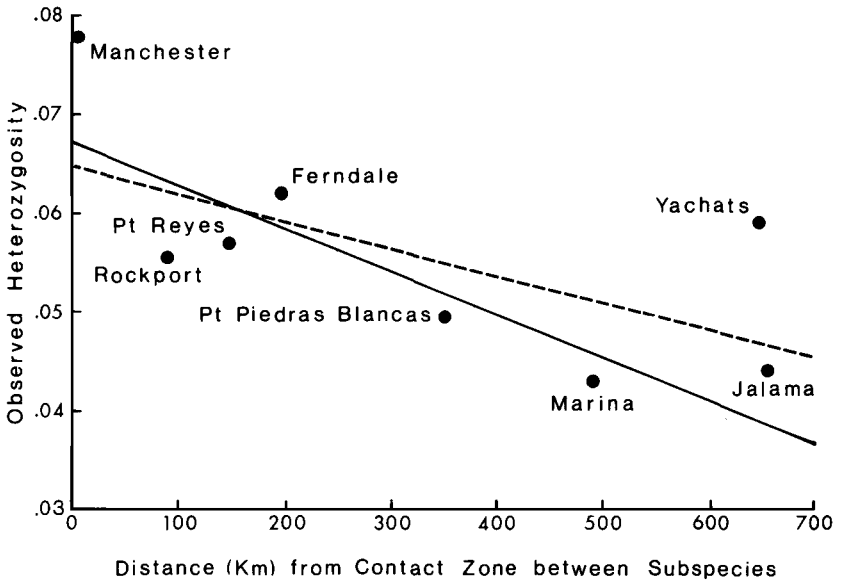

Fig. 2. Linear regressions of average observed heterozygosity on distance of populations north and south of Manchester, California. The value for Yachats, Oregon is included in the calculation of the dashed regression line and is excluded from that of the solid line.

recognized southern boundary of the zone of intergradation between *Z. l. nuttalli* and *Z. l. pugetensis* (Banks 1964, Mewaldt et al. 1968). If the population at Yachats, Oregon is included in the regression analysis, then $y = 0.0659 - 0.00003x$, and the slope is significantly different from zero ($t_{6df} = -2.2477$, $P < 0.05$). If Yachats is excluded from the analysis, then $y = 0.0680 - 0.000045x$ ($t_{5df} = -3.6614$, $P < 0.01$). The slopes of these two regressions are significantly different from zero, but, in addition, the relationship between $\bar{H}$ and distance from the contact zone does not appear to be linear.

Thus, in a preliminary attempt to examine this relationship further, the heterozygosity data were fitted by means of a least squares regression to an exponential decay curve of the form $\bar{H} = c_1 e^{\pm c_2 x}$. In linear form, the constants of integration, c_1 and c_2, become the y intercept, a, and the slope of the regression line, b, respectively. The fit is now only slightly better, as shown in Fig. 3, where the data again have been analyzed either with or without the value of $\bar{H}$ for Yachats, Oregon. If the value of $\bar{H}$ for Yachats is included, then $y = e^{-0.0038x - 3.813}$ ($t_{6df} = -2.030$, $P < 0.05$). Without the value of $\bar{H}$ for Yachats, $y = e^{-0.0062x - 3.466}$ ($t_{5df} = -5.003$, $P < 0.01$). In plotting y as a function of distance from Manchester, California (Fig. 3), a constant value of 0.043 has been added to each value of y. Thus, the curves asymptotically approach this value rather than zero. The rationale for doing this is that the average value of $\bar{H}$ for passerine birds is estimated to be 0.043 (Barrowclough and Corbin 1978) and not zero.

Discussion

The objectives of this paper are: (1) to present data showing that values of average heterozygosity, $\bar{H}$, within the distributions of two subspecies of the White-crowned

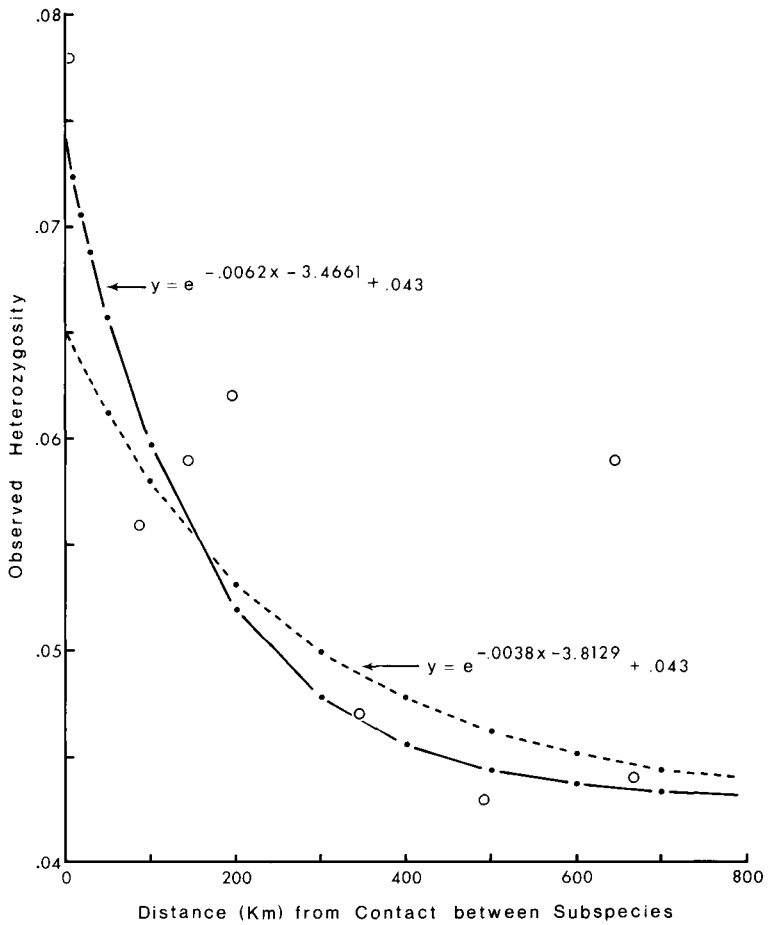

Fig. 3. Curvilinear regressions of average observed heterozygosity on distance of populations north and south of Manchester, California, as fitted to an exponential decay curve that asymptotically approaches the value of 0.043, the average heterozygosity for birds (Barrowclough and Corbin 1978). The value for Yachats, Oregon is included in the calculation of the dashed curve and excluded from that of the solid curve. Populations are positioned as in Fig. 2.

Sparrow in California and Oregon are highest in, and significantly correlated with distance from, the point of contact between the subspecies, and (2) to suggest that $\bar{H}$ may be used as an objective index of the midpoint of zones of intergradation between subspecies and thereby serve to delineate their geographic distributions.

Heterozygosity may be calculated in either of two ways, one of these based on the number of heterozygous loci observed in each individual of a population sample and the other based on the frequency of the alleles in the population sample. The former is possible only if each individual is assayed for all of the loci surveyed in the genetic analysis, but, of the two estimates of $\bar{H}$, it is the more accurate and no assumptions are made about the breeding structure of the deme from which the population sample is drawn. That is, values of $\bar{H}$ based on allelic frequencies and calculated as described above in the Methods Section are accurate only if individuals in the population are in Hardy-Weinberg equilibrium, which is not true for any of these White-

crowned Sparrow populations. Although both methods are subject to errors resulting from the Wahlund effect (Wahlund 1928), the potential error inherent in mixing samples from different demes has been minimized in this study, because all individuals from a given locality were obtained from a breeding population confined to 8 ha or less.

A second major reason for using observed values of $\bar{H}$ in this study is to reduce the variance associated with this parameter. Because individual heterozygosity, h, is calculated here as the ratio of the number of heterozygous loci in an individual to the total number of loci assayed in that individual, h varies from zero to a value equal to the ratio of: (number of polymorphic loci in the population sample) ÷ (number of polymorphic loci + number of monomorphic loci). This maximum value would be found if an individual were heterozygous at every polymorphic locus. For this study, the maximum value possible would be 13/44 = 0.295. No such individual was found, and the maximum h observed was 0.146 (6/41). Because the average observed heterozygosity, $\bar{H}$, is the mean of the individual values of h, its variance can be reduced by increasing the sample size. By contrast, the variance around calculated $\bar{H}$, based on allelic frequency data, can be reduced only by increasing the number of loci sampled. This places almost insurmountable constraints on its use in any analysis wherein population variance is a confounding factor, as is true in this study, wherein variation in $\bar{H}$ is being associated with the geographic distribution of the subspecies.

In theory, heterozygosity in a population will be maximized at any given locus when the alleles of that locus, regardless of their number, are equal in frequency. When the number of alleles at a locus is less than 5 or 6, changes in allelic number will have some effect on heterozygosity, but the value of $\bar{H}$ is relatively insensitive to changes in the number of alleles when the allelic frequencies are not equal. Indeed, $\bar{H}$ is least perturbed by changes in allelic number when one allele is at frequencies above 0.9 and additional alleles are at low frequencies.

Because $\bar{H}$ is maximized when either (1) allelic frequencies at loci are equalized (for values of $\bar{H}$ based on allelic frequencies) or (2) when the average number of heterozyogous loci per individual is maximized, are there reasons to expect these conditions to be found in particular regions of a species' geographic distribution? This can be rephrased to ask under what conditions will there be a maximum exchange between populations of alleles that are unique, thereby resulting in steep clines of allelic frequencies, which will result in the convergence of allelic frequencies at variable loci. Clearly, these conditions are not going to be found at the geographic margins of a species' distribution, where genetic exchange is greatly diminished and where there may be little or no migration between isolated, marginal populations, nor will these conditions be found within central areas of the distribution, where panmixia prevails. In fact, the conditions leading to maximum values of $\bar{H}$ are precisely those found within zones of contact between subspecies, and, on average, subspecies in secondary contact will be more highly differentiated than those in primary contact. In these zones, allelic frequencies will be changing rapidly if alleles are maintained at different frequencies in the respective subspecies. Given this condition, allelic frequencies will converge toward similar values somewhere in the contact zone, because as the frequency of one allele falls, that of another rises. Simultaneously, this increases the probability that an individual will be heterozygous

at any given polymorphic locus. The net result will be an increase of $\bar{H}$ in the contact zone.

As a general hypothesis, the value of $\bar{H}$ should increase in zones of contact as a function of the following conditions: (1) the greater the degree of genetic differentiation between the subspecies, the higher will be the maximum value of $\bar{H}$; (2) the more nearly equal the frequency of alleles at a locus, the higher the value of $\bar{H}$; (3) the greater the number of loci that possess alleles of nearly equal frequencies at the same point within the contact zone, the higher the value of $\bar{H}$; and (4) the larger the proportion of polymorphic loci, the larger the value of $\bar{H}$. Obviously, various combinations of these conditions may be realized within any given zone of contact. Thus, the magnitude of the rise in $\bar{H}$ within a contact zone probably will vary considerably among zones of intergradation, whether primary or secondary in nature.

When considering changes in $\bar{H}$ in zones of contact between well-differentiated subspecies, as measured by F_{st} (Cockerham 1973) or genic contingency χ^2 values (Workman and Niswander 1970), the rise in $\bar{H}$ over the average value for the subspecies may be significant. This is apparently true for the contact zone between *nuttalli* and *pugetensis*. Not only is there a highly significant relationship between observed $\bar{H}$ and the distance of the population from the contact zone (Figs. 2 and 3), but also the maximum observed value of $\bar{H}$ (0.078 ± 0.008 at Manchester, California) is significantly different from the value of $\bar{H}$ for the subspecies *nuttalli* (0.049 ± 0.003, based on values of samples from Jalama, Pt. Piedras Blancas, Marina, and Pt. Reyes, California) ($t_{82} = 3.683$, $P < 0.001$). Because only one population sample was obtained from geographic areas that are unequivocally occupied by *pugetensis* (Yachats, Oregon), the comparison is not made in the other direction.

I submit, for consideration, that $\bar{H}$ is a kind of common denominator in comparing subspecies that makes it possible to summarize the changes occurring at many loci simultaneously. No such common denominator exists for other kinds of taxonomic studies that rely on various other criteria to distinguish taxa. Like these other character systems employed by systematists, however, individual clines in allelic frequencies are not likely to be concordant if selective regimes are different for the different loci examined. Some clines will be broader, some steeper, and at some loci there may not be any changes in allelic frequencies across the zone of intergradation. Nevertheless, $\bar{H}$ provides a distillation of these various changes, a condensation of the disparate measures into a single value that reflects the average flux of allelic combinations, of coadapted gene complexes, across the contact zone. As such, $\bar{H}$ may be a superior measure of the contact point between genetically differentiated populations, which the taxonomist designates as subspecies. It is understood here that the contact point or area will fall *within* the zone of intergradation and is not synonymous with it.

Turning now to a review of past and current viewpoints regarding the location of the transition zone between *pugetensis* and *nuttalli,* we find among the many studies of this species only four that address this issue directly. In his paper describing *Z. l. pugetensis* as a new subspecies, Grinnell (1928) stated that *pugetensis* was "intergrading somewhere along there [coast of Mendocino] with *nuttalli*." This statement of his is vague to the extent that it refers to a stretch of coastline approximately 150 km in length. Judging from the current holdings in the collection at the Museum

of Vertebrate Zoology, University of California, Berkeley, to which Grinnell undoubtedly referred, however, his statement may have been based upon the inclusion of a small sample of birds taken from Gualala, at the southern border of Mendocino County.

An analysis of migration data led Blanchard (1941) to conclude that a transition zone between migratory and nonmigratory individuals extended from Coquille, Oregon southward to Waddington, Humboldt County, California. The southern edge of the zone proposed by her falls about 75 km north of the northernmost point recognized by Grinnell.

In his revision of the species, Banks (1964: 39) set the dividing point between *pugetensis* and *nuttalli* "in the vicinity of Cape Medocino, in northern California, between my Humboldt and Mendocino populations." This point falls roughly midway between the southern boundary of Blanchard's proposed zone and the northern edge of Grinnell's zone. Although Banks (1964) went on to state that intergradation is most obvious to the north of this point, the mensural data presented do not support this statement. Rather, they show that clines of bill length, toe length, and tarsus length change direction at Humboldt County to the north and at Mendocino County to the south. A cline in the body weights of males does not change direction until farther south of Mendocino, reversing direction between the Sonoma County sample to the north and the Marin County sample to the south. This area lies about 250 km south of the division point chosen by Banks, but the zone of intergradation, as judged by clinal variation in male weights (one of the two characters used by Banks to distinguish individuals of *pugetensis* from those of *nuttalli*), clearly extends at least as far south as his Sonoma County sample and perhaps should include the Marin County samples as well.

The findings of Mewaldt et al. (1968) placed the zone of intergradation between Albion, Mendocino County, California to the south [a locality about 5 km south of Bank's (1964) southernmost Mendocino County sample] and Capetown, Humboldt County, California to the north, at or slightly north of Bank's dividing point. The width of this transition zone is approximately 147 km, following the coastline; its location is shown in Fig. 1.

Significant new evidence relevant to the identification of localities that should be included in the zone of intergradation is provided by the electrophoretic analyses of the 13 polymorphic loci examined here. The statistical analysis of the allelic frequency data by means of Wrightian F-statistics, the results of which are summarized in Fig. 1, show that a significant discontinuity in allelic and genotypic frequency distributions exists between Manchester, Mendocino County, California to the north, and Pt. Reyes, Marin County to the south (Fig. 1). Manchester lies approximately 38 km south of Albion, California. Furthermore, the genetic constitution of the Manchester population is not significantly different from that of the Rockport population, about 56 km north of Albion (Fig. 1) and near the middle of the zone of intergradation proposed by Mewaldt et al. (1968).

On the basis of this evidence from the analysis of the genetic data, it appears that the zone of intergradation between *pugetensis* and *nuttalli* extends at least another 35 km southward from Albion, California, to include the breeding population of Manchester and vicinity. Indeed I shall argue below that this area falls at the midpoint of the zone and not at its southern limit.

Looking now at the north end of the zone of intergradation with reference to the

distributions of allelic and genotypic frequencies, there are no breaks or discontinuities at any point between Rockport, California and Yachats, Oregon. In fact, the population at Rockport forms part of a genetically homogeneous subset that also includes populations at Ferndale, Humboldt County, California and Yachats, Oregon.

Taken together, these various lines of evidence indicate that a marked shift in phenotype from *pugetensis* to *nuttalli* occurs and is readily recognized along the southern coast of Mendocino County in the vicinity of Albion and Manchester. Patently, the precision of any such statement is, in part, a function of the sampling density used in the respective studies and the degree of identity of localities sampled. Therefore, whether this shift occurs at Mendocino, Albion, or Manchester, California appears to be a function of sampling procedures and not of fundamental biological differences in the character suites examined.

On the other hand, Banks' (1964) dividing point appears to coincide with the northern edge of the zone of intergradation, as proposed by Mewaldt et al. (1968). But what is the biological significance of this dividing point? It is that point in the clines of tarsal length and the body weight of males at which the first obvious change in slope occurs, passing from regions occupied by *pugetensis* into the zone of intergradation. This dividing point does not coincide with that point *within* the zone of intergradation where the average genetic composition shifts from being more like *pugetensis* to more like *nuttalli*. Rather, Banks' dividing point separates individuals of the *pugetensis* phenotype from those that are intergrades. As such, the systematic consequence is to place all intermediate forms into the subspecies *nuttalli*. In the absence of more recent findings (Mewaldt et al. 1968, and results presented here), there was no acceptable alternative way to deal with this taxonomic problem, as there were no other unambiguous division points between the races.

Now, however, the findings of Mewaldt et al. (1968), coupled with my F_{st} analyses of the genetic variation in these subspecies, unequivocally indicate that breeding individuals from as far south along the coast as Albion or Manchester are more similar to *pugetensis* than to *nuttalli*.

We may then ask whether setting a boundary between *pugetensis* and *nuttalli* at Manchester or Albion would be fundamentally different from setting it at Cape Mendocino, 145–160 km to the north. It would be if, and only if, the Manchester-Albion region falls within the zone of intergradation and not at its southern terminus. I have argued above that the average observed heterozygosity, $\bar{H}$, may be viewed as a parameter by which to measure the flux of coadapted gene complexes across a zone of intergradation when subspecies are genetically differentiated. Furthermore, in theory, $\bar{H}$ should reach its maximum value at the midpoint of this transition zone. Therefore, based on the data presented here (Table 1, Figs. 1–3), wherein it is unambiguous that $\bar{H}$ reaches a maximum value at Manchester, California, I submit that the Manchester-Albion area is in fact at or near the midpoint of the zone of intergradation between *pugetensis* and *nuttalli*.

If the zone of intergradation is symmetrical with respect to gene flow and if the Manchester-Albion area is at or near the midpoint of the zone, then the zone of intergradation should extend as far south from Manchester-Albion as it does north. Taking Cape Mendocino as its northern edge, then the zone would extend as far south as Bodega Head in Sonoma County or Pt. Reyes in Marin County. [Although I do not place much weight on the observation, there is an independent measure of

these distances that can be obtained from the values of the curvilinear regressions presented in the Methods Section. As applied to radio isotope decay, the reciprocal of the slope, b, in those equations is equal to the half-life of the decay time. As used here, $1/b$ might be thought of as ½ the distance from the midpoint of the zone of intergradation required to diminish $\bar{H}$ to average values for the subspecies. As such, $1/b$ might correspond roughly to the distance from the midpoint to the recognizable boundary of the zone of intergradation. Using the value of $b = 0.0062$ obtained for the best fitting curve (Fig. 3), its reciprocal equals 164 km which is only 1 km less than the distance between Albion and Capetown.]

What evidence exists for believing that the zone extends as far south as the Sonoma County-Marin County border? Mewaldt et al. (1968) noted that birds from the zone of intergradation should be larger on average than birds from outside the zone. In both their study and that of Banks (1964) the weights of males were decidedly larger from the localities of Bolinas, Fort Ross, Albion, and Capetown (Mewaldt et al. 1968) and Humboldt County, Mendocino County, Sonoma County, and Marin County (Banks 1964). In the latter three counties tarsal lengths were markedly longer (Banks 1964). The curve for heavy prenuptial molt intersects that for light prenuptial molt at Fort Ross, Sonoma County, but extends into Marin County at Bolinas (Mewaldt et al. 1968). Mewaldt and King (1978) found the coefficient of variation of molt duration to be highest at Albion, while it decreased markedly north of Cape Town and fell off more gradually south of Bolinas in Marin County. Thus, it seems that the same arguments that have been used to set Cape Mendocino as the northern boundary of the zone of intergradation can be applied as well to setting the southern boundary of the zone in the vicinity of northern Marin County or southern Sonoma County, and, if this is true, then the Manchester-Albion area does indeed fall near the middle of the zone of intergradation of *pugetensis* with *nuttalli*.

It is premature to make a general statement that heterozygosity can be expected to increase in all zones of intergradation between subspecies. At least one example is already known in which two phenotypes of the Yellow-rumped Warbler (*Dendroica coronata*), previously recognized as species but now designated as subspecies, have not differentiated genetically and therefore values of $\bar{H}$ do not change significantly in the zone of intergradation (Barrowclough 1980). Thus, in those instances where populations are not well differentiated genetically, as measured by significant differences or variance in allelic frequencies, heterozygosity probably cannot be used as an indicator of the point of contact between them. Obviously, estimates of genic heterzygosity must be obtained from more zones of both primary and secondary intergradation, in the manner presented here, before any general statement can be made concerning the efficacy of using heterozygosity as an index to limits of geographic distributions between subspecies.

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