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GENETIC AND PHENOTYPIC DIFFERENTIATION IN A WOOD WARBLER (GENUS *DENDROICA*) HYBRID ZONE

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ABSTRACT.—Electrophoresis and mathematical models of clines were used to investigate the extent of genetic differentiation and the magnitudes of the evolutionary forces acting in a *Dendroica c. coronata–D. c. auduboni* (Aves: Parulidae) hybrid zone in the Canadian Rocky Mountains. Estimates of the genetic distance between the two taxa are comparable to the degree of genetic differentiation found among subspecies of other passerines. The normalized among-population component of genetic variance, as measured by F_{st}, is of the order of 0.01. This is similar to that found among populations of other species of birds. Thus, there is no evidence for extensive genetic differentiation or a "genetic revolution" between these two forms at the structural gene level.

It is difficult to discriminate among alternative models for the origin of the phenotypic differences between the two taxa, but an allopatric origin in geographically isolated refugia during the Pleistocene is the most parsimonious interpretation of the available data. Secondary contact probably occurred approximately 7,500 yr ago. An investigation of the evolutionary forces responsible for the phenotypic differences between the two taxa suggests that the current width of the hybrid zone is completely consistent with a dynamic cline involving the diffusion of neutral alleles. If, instead, the cline is currently in gene flow-selection equilibrium, then the magnitude of selection against the traits of one morph in the range of the other may only be of the order of 10^{-4}. In the absence of knowledge about the degree of dominance of alleles coding for the variable phenotypic characters, however, discrimination among alternative cline models is difficult. In any case, it is not necessary to invoke the existence of strong selective forces to explain the cline through this hybrid zone. Any selection for positive assortative mating would not be deterministic; hence, these taxa should be treated as subspecies. *Received 25 January 1980, accepted 19 May 1980.*

HYBRID zones have long been interesting to evolutionary biologists, because they may offer a chance to examine a potentially important stage in the process of speciation (Mayr 1963). Until recently such examination was largely phenomenological. With the development of newer quantitative techniques and simple mathematical models, however, it has become possible to attempt analyses of the types and magnitudes of evolutionary processes involved in hybrid zones. Electrophoretic analysis of population samples has been shown to be a useful tool for analyzing the degree of differentiation among avian taxa (Barrowclough and Corbin 1978, Corbin et al. 1979, Avise et al. 1980), and Endler (1977) has summarized some of the simple cline theory models for the analysis of selection and gene flow. These two techniques have been employed in this study of an avian hybrid zone in an attempt to discriminate,

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TABLE 1. List of 32 genetic loci examined for five populations of *Dendroica coronata*. Abbreviation is in parentheses.

Acid phosphatase	(AcP)
Creatine kinase	(CK)
Esterase	(EST-1)
Glutamate dehydrogenase	(GDH)
Glutamate oxaloacetate transaminase	(GOT-1, GOT-2)
Glycerophosphate dehydrogenase	(GPDH)
Hemoglobin	(Hgb-1, Hgb-2)
Isocitrate dehydrogenase	(IDH)
Lactate dehydrogenase	(LDH-1, LDH-2)
Malate dehydrogenase	(MDH-1, MDH-2)
Malic enzyme	(ME)
Mannose phosphate isomerase	(MPI-1, MPI-2)
Muscle protein	(Pro-m1, Pro-m2, Pro-m3, Pro-m4)
Peptidase	(Pep A, Pep B, Pep C)
Phosphoglucose isomerase	(PGI)
Phosphoglucomutase	(PGM-1, PGM-2)
Plasma protein	(Pro-pl)
Sorbitol dehydrogenase	(SDH-1, SDH-2, SDH-3)
Superoxide dismutase	(SOD)

so far as current knowledge and techniques allow, among the evolutionary forces that might be responsible for the current status and to compare the results with the forces and events generally postulated for such zones. The application of cline theory to problems of geographic variation in birds has also been used recently by Gill (1973) and Wunderle (1980).

The hybrid zone between two subspecies of the Yellow-rumped Warbler, *Dendroica c. coronata* and *D. c. auduboni*, was chosen for this analysis. These taxa are phenotypically quite distinct and were recognized until recently as two species (A.O.U. Comm. Classif. Nomen. 1973). The contact between these two taxa in British Columbia was thoroughly described and qualitatively analyzed by Hubbard (1967, 1969). One of the three areas of contact described by Hubbard was reinvestigated in this study. This was the Pine Pass transect between Dawson Creek and Prince George, British Columbia.

METHODS

Electrophoresis and mathematical modeling were used to analyze individuals of several breeding populations of *Dendroica coronata* that were collected during the spring and summers of 1976, 1977, and 1978. Specimens of *D. c. auduboni* were collected in the Beartooth Mountains of southern Montana and in the Cascade Mountains of northern Washington. Specimens from the hybrid zone were collected at Chetwynd and at McLeod Lake, British Columbia, on the eastern and western sides of Pine Pass, respectively. Specimens of *D. c. coronata* were collected in northern Minnesota. Subsequently, populations will be referred to by the name of the collecting locality. Sample sizes were: Beartooth, 21; Cascade, 23; McLeod, 13; Chetwynd, 21; Minnesota, 27. Samples of liver, breast muscle, plasma, and red cells were taken from each specimen and stored in liquid nitrogen. Prior to electrophoresis, proteins from the nonblood tissues were extracted at 4°C in a solution of phenoxyethanol, sucrose, and phosphate buffer (Nakanishi et al. 1969). Genetic variation and differentiation were examined by starch gel electrophoresis and specific isozyme staining, as described by Barrowclough and Corbin (1978) and Barrowclough et al. (in prep.).

Heterozygosity.—Individuals were scored as to genotype at the 32 loci listed in Table 1. Protein morphs with differing electrophoretic mobilities at a given locus were inferred to be the products of different alleles. Heterozygosity was calculated from the data in two ways. First, allelic frequencies were computed from genotypic frequencies, and the expected heterozygosity was computed for each locus. Under the assumption of Hardy-Weinberg equilibrium, this was estimated as $\hat{h} = 1.0 - \Sigma \hat{p}^2_i$, where $\hat{p}_i$ is the estimated frequency of the i^{th} allele in the population. The average across loci was then obtained. In a second method, heterozygosity was calculated as the average of the observed frequencies of heterozygotes at each locus.

Genetic differentiation.—The methods of Nei (1978), and Rogers (1972) were used to calculate the genetic distances between populations. These methods require information only about allelic frequencies and sample sizes. Additional analyses were carried out using the procedures suggested by Cockerham (1969, 1973). These involve the estimation of components of genetic variance for the allele at highest frequency at each variable locus. Both a weighted and an unweighted method have been described, but it is not known which of the two is better (Cockerham 1973); the arithmetically simpler, unweighted method is used here. For each variable locus, variance components of genotypic frequencies within individuals, among individuals, and among populations were estimated, and statistics reflecting the population structure were calculated from these. These statistics, originally devised by Wright (1951), are designated F_{is}, F_{st}, and F_{it}. F_{is} is a measure of the departure of genotypic frequencies from Hardy-Weinberg equilibrium that is attributable to a given mating system within a population. It may be either positive or negative, with a negative value reflecting fewer homozygotes than would be expected on the basis of random mating. A positive value reflects an excess of homozygotes. F_{st} is a measure of the departure from panmixia among populations, reflecting local differentiation into subpopulations or demes. F_{it} combines the effects of both nonrandom mating and geographical subdivision of the total population. For the data treated here, overall $\hat{F}_{is}$, $\hat{F}_{st}$, and $\hat{F}_{it}$ were estimated by their mean values, averaged over all polymorphic loci. Chi-square tests of significance of these estimates were conducted as described by Cockerham (1973). For each locus the tests include a test of $\hat{F}_{st} = 0$ with $M - 1$ degrees of freedom, where M is the number of populations, and a test of $\hat{F}_{is} = 0$ with one degree of freedom. In order to ascertain whether the average $\hat{F}$ values were significantly different from zero, t-tests were performed.

In addition to the *Dendroica coronata* data reported here, the avian electrophoretic literature was searched for studies in which genotypic frequencies were reported for one or more variable loci in two or more natural populations. The genotypic frequencies from these studies were used to estimate the extent of genetic differentiation according to the methods described above.

Phenotypic differentiation.—All individuals collected in the two hybrid zone localities in British Columbia were prepared as museum study-skin specimens. A hybrid index score was assigned to each specimen for each of six characters (throat color, auricular color, presence of a postocular line, quantity of white in the wingbar, and quantity of white in the tail) using methods described by Hubbard (1967). Before values were assigned to any of these specimens, however, some of Hubbard's specimens from the same hybrid localities were examined in order to gain familiarity with birds of known scores. After the specimens were assigned individual character scores, an overall hybrid index was calculated for each bird as the average of the six individual character scores. This overall index varies from zero to two; zero indicates a "pure" *D. c. coronata* type, and two indicates a "pure" *D. c. auduboni* type.

The mean hybrid index for each of the six characters, as well as an overall hybrid index, was computed as the average across individuals for each of the two hybrid zone populations. Lines were fitted to these data to describe the change in hybrid index with distance. Then the hybrid zone width was estimated as the distance between the points where populations would theoretically take on mean scores of 0.4 and 1.6. This is the distance over which a single-locus, additive-gene cline would change in allelic frequency from 0.2 to 0.8, and hence conforms to the definition of cline width used in the equations of population genetics (May et al. 1975).

RESULTS

Heterozygosity.—Of the 32 genetic loci examined, 24 were monomorphic across the 5 population samples studied. The allelic frequencies for the eight remaining loci are reported in Table 2 for the five populations. Observed and expected heterozygosities for the five populations were computed. The mean expected and the mean observed heterozygosities, calculated over populations, are 0.037 and 0.031, respectively. For each population, chi-square values were computed to test for departures of the number of observed heterozygotes at each locus from the expected number. These values were $\chi^2_4 = 1.592$, $\chi^2_5 = 0.507$, $\chi^2_4 = 4.263$, $\chi^2_3 = 4.399$, and $\chi^2_3 = 0.340$ for the Beartooth, Cascade, McLeod, Chetwynd, and Minnesota population samples, respectively. None of these chi-square values is significant at the 0.05 level.

Genetic differentiation.—There are no simple trends of allelic frequencies with

TABLE 2. Estimates of allelic frequencies, at variable loci, for five populations of *Dendroica coronata*.

Locus	Allele	Beartooth	Cascade	McLeod	Chetwynd	Minnesota
CK[a]	A					0.019
	B	0.976	1.000	1.000	1.000	0.982
	C	0.024				
LDH-2	A					0.019
	B	1.000	1.000	1.000	1.000	0.982
Pep A	A	0.095	0.152	0.150	0.100	
	B	0.905	0.848	0.850	0.900	1.000
Pep B	A	0.048	0.068	0.039	0.046	0.063
	B	0.952	0.932	0.962	0.955	0.896
	C					0.042
Pep C	A	0.095	0.044	0.039	0.048	0.150
	B	0.881	0.935	0.885	0.905	0.800
	C	0.024	0.022	0.077	0.048	0.050
PGM-1	A	1.000	1.000	1.000	0.976	1.000
	B				0.024	
PGM-2	A			0.039		
	B	0.976	0.913	0.923	1.000	1.000
	C	0.024	0.044	0.039		
	D		0.044			
Pro-pl	A	0.310	0.205	0.539	0.318	0.636
	B	0.667	0.750	0.462	0.682	0.364
	C	0.024	0.046			

[a] Isozymes and alleles are labelled according to mobility. For a class of isozymes, the isozyme with the greatest cathodal mobility is assigned the number 1; consecutively less mobile loci are asigned numbers 2, 3, etc. The nomenclature for multiple alleles at a locus is analogous. The most cathodal allele is assigned the letter A; consecutively less mobile alleles are assigned the letters B, C, etc.

distance. For instance, correlations of frequency of the most common allele on distance are insignificant at the 0.05 level for all eight variable loci. Furthermore, a chi-square test for heterogeneity of allelic frequencies among populations (Workman and Niswander 1970) was significant at only one of these eight variable loci, Pro-pl ($\chi^2_8 = 18.023$, $P < 0.05$).

Because sample sizes at individual loci are not large, descriptive statistics and analyses based on multiple loci should be emphasized. The genetic distances between the five populations sampled, using Nei's (1978) and Rogers' (1972) statistics, are reported in Table 3. The values range from −0.0011 to 0.0081 for Nei's distance, and from 0.0059 to 0.0349 for Rogers' distance. As Nei pointed out, negative genetic distances can be obtained for populations that are very similar in allelic frequencies, due to sampling error.

A second class of multilocus descriptive statistics that reflects genetic differentiation is the F statistics (Crow and Kimura 1970). F_{is} corresponds to the well-known individual inbreeding coefficient; F_{st}, however, may not be so familiar. It is an index of the amount of population subdivision—a value of one indicates fixation of

TABLE 3. Matrix of genetic distance estimates between five populations of *Dendroica coronata*. Distances computed by the methods of Rogers (1972) (above diagonal) and Nei (1978) (below diagonal).

Population	1	2	3	4	5
1. Beartooth	—	0.0128	0.0170	0.0059	0.0249
2. Cascade	−0.0002	—	0.0184	0.0126	0.0349
3. McLeod	0.0008	0.0029	—	0.0164	0.0213
4. Chetwynd	−0.0011	−0.0004	0.0006	—	0.0265
5. Minnesota	0.0038	0.0081	0.0003	0.0039	—

TABLE 4. Components of genetic variance (Cockerham 1969, 1973) at eight electrophoretically polymorphic loci in *Dendroica coronata*. Mean and one standard error are indicated for F statistics.

Locus	CK	LDH-2	Pep A	Pep B	Pep C	PGM-1	PGM-2	Pro-pl	$\hat{F}$
Allelic frequency	0.990	0.995	0.903	0.934	0.883	0.995	0.957	0.615	
Number of populations	5	5	5	5	5	5	5	5	
Number of individuals	105	105	93	91	98	105	82	78	
$\hat{F}_{is}$	0.0028	0.0055	0.2618	−0.0534	0.2700	0.0000	−0.0498	0.2062	0.0804 ± 0.0496
$\hat{F}_{st}$	−0.0110	−0.0112	0.0065	−0.0176	−0.0099	−0.0015	0.0115	0.0710	0.0047 ± 0.0101
$\hat{F}_{it}$	−0.0082	−0.0056	0.2666	−0.0719	0.2628	−0.0015	−0.0377	0.2625	0.0834 ± 0.0535

TABLE 5. Components of electrophoretically detected genetic variance in seven species of birds. F values reported are the means across loci.

Species	Locality	Number of individuals	Number of populations	Number of loci	$\hat{F}_{is}$	$\hat{F}_{st}$	$\hat{F}_{it}$	References
Dendragapus obscurus	British Columbia	389	3	1	−0.028	0.004	−0.024	Redfield (1974)
Zonotrichia capensis	Argentina	195	5	4	0.195	0.022	0.215	Handford and Nottebohm (1976)
Zonotrichia leucophrys	North America	227	9	6	0.000	0.015[a] 0.077[b]*	0.091	Baker (1975)
Dendroica coronata	North America	105	5	8	0.080	0.005	0.083	This study
Icterus galbula	North America	213	8	2	0.312	0.027	0.325	Corbin et al. (1979)
Aplonis cantoroides	New Guinea	91	4	2	0.034	0.097	0.124	Corbin et al. (1974)
Aplonis metallica	New Guinea	354	15	2	0.024	0.048	0.075	Corbin et al. (1974)

[a] Within geographical regions; * $P < 0.01$.

[b] Between geographical regions.

alternate alleles among populations, while a value of zero indicates an absence of subdivision. The results of an F-statistic analysis of the components of genetic variance for the eight variable loci are given in Table 4. The three $\hat{F}$ statistics are shown for each locus, and the means across loci and their empirical standard errors are also given. None of the three overall average $\hat{F}$ statistics is significantly different from zero at the 0.05 level (t-test).

In Table 5 the average values of $\hat{F}_{is}$, $\hat{F}_{st}$, and $\hat{F}_{it}$ across loci are reported for the seven species of birds for which adequate electrophoretic data were available in the literature for my analysis. In the case of *Dendragapus obscurus,* data for 3 yr were reported (Redfield 1974). For this analysis, however, only the 1971 data, the year with the largest sample sizes, were used. In most cases the number of variable loci examined in the studies was small. This, along with the fact that there tends to be considerable variance of the estimates of F components among loci, resulted in relatively large standard errors associated with the means reported in Table 5. However, in the analysis of the study with the largest number of variable loci, Baker's (1975) work on *Zonotrichia leucophrys,* $\hat{F}_{st}$ among regions equals 0.077 ± 0.015. The results of a t-test on this estimate ($t_5 = 5.133$, $P < 0.01$) indicate that a portion of the genetic variance in that species is distributed between its California and Colorado ranges. None of the other estimates of F components was significantly different from zero.

Phenotypic differentiation.—The hybrid index that Hubbard (1967) developed for this warbler complex is simple and straightforward; only three states were recognized for each character. Consequently, as with other avian hybrid complexes (Corbin and Barrowclough 1977), it proved easy to reproduce the scores Hubbard had obtained for his specimens. For the McLeod Lake and Chetwynd localities, Hubbard's specimens' overall mean hybrid indices were 1.564 ± 0.081 (empirical standard error across individuals) and 0.334 ± 0.071, respectively. For the same localities, the corresponding figures for the specimens collected in this study were 1.617 ± 0.058, and 0.239 ± 0.042. In neither case are these means significantly different ($t_{24} = 0.533$, and $t_{30} = 1.223$).

Data from this study were pooled with those of Hubbard (unpubl.) to estimate the width of the hybrid zone. The width based upon the overall hybrid index was 147.3 km. The among-character standard deviation for width was 16.3 km.

DISCUSSION

Dendroica coronata is insectivorous and not readily bred in captivity. Consequently, breeding studies have not been performed to ascertain the Mendelian nature of the various electrophoretic mobility classes. Two lines of evidence support the supposition that the variation studied is genetic, however. First, the pattern of biochemical variation conforms with the pattern of variation established to be genetic by breeding studies in *Drosophila* and a few other organisms. Second, the within-population variation is in agreement with genotypic frequencies expected on the basis of Hardy-Weinberg equilibria. It seems unlikely that nongenetic variation would closely approximate genetic expectations. Thus, it seems reasonable to conclude that the electrophoretic variation observed is genetic in origin.

Heterozygosity.—The estimation of the amount of genic heterozygosity in natural populations has been the subject of intense investigation following the initial reports of Hubby and Lewontin (1966) and Lewontin and Hubby (1966). This is due to the

TABLE 6. Summary of the degree of genetic differentiation (Nei 1978), as a function of taxonomic level, among avian populations.[a]

Taxonomic level:	Local populations	Subspecies	Species	Genera	Families
Mean genetic distance	0.0024	0.0048	0.0440	0.2136	0.6829
Standard error	0.0003	0.0005	0.0026	0.0141	0.0304
Standard deviation	0.0028	0.0049	0.0221	0.1659	0.1970
Range	−0.0014–0.0125	−0.0014–0.0214	0.0078–0.1267	0.0126–1.2140	0.3365–1.2140
Sample size	113	86	71	139	42

[a] Based on the studies of Corbin et al. (1974), Baker (1975), Handford and Nottebohm (1976), and Smith and Zimmerman (1976), Barrowclough and Corbin (1978), Corbin et al. (1979), Barrowclough et al. (unpubl.), Corbin (unpubl.).

fact that genetic variation is the raw material of evolutionary processes and consequently is an important variable in population genetics theory. These papers brought to the attention of researchers a method for estimating the magnitude of this variation. The quantity of such variation, based on electrophoretic estimates, has been reviewed in recent years by Powell (1975) and by Nevo (1978). In addition, Barrowclough and Corbin (1978) reviewed the results for avian studies in particular. The observed heterozygosity reported here for *Dendroica coronata,* 0.031, is in the same range as the average observed heterozygosity in other bird species examined to date, 0.043 ± 0.014 (empirical SD). Furthermore, as Barrowclough and Corbin (1978) pointed out, this estimate of mean heterozygosity for birds is approximately the same as that of other vertebrate taxa, 0.049 ± 0.037 (SD) (Nevo 1978). Nevertheless, because the fraction of the total genic variation uncovered by electrophoresis is in doubt (Singh et al. 1976), the actual significance of estimates such as these remains questionable.

Genetic differentiation.—The mean standard genetic distance (Nei 1978) between *D. c. coronata* and *D. c. auduboni* population samples, exclusive of the hybrid zone samples, is 0.0060 ± 0.0022 (SE). In Table 6 the frequency distributions of genetic distance among populations of birds separated at various taxonomic levels are summarized. This table is based on eight different studies with sample sizes that, while still not as large as one might like, are sufficient for some confidence to be placed in the parameters extracted from the distributions. For this analysis, all genetic distances, including those of the older studies, have been recomputed using Nei's (1978) recent modification that removes bias due to small sample sizes. The genetic distance between *D. c. coronata* and *D. c. auduboni* is less than one standard deviation from the mean of the distribution for subspecies but lies below the range of observed values of interspecific genetic distances. Although there is no reason to assume that a causal link exists between genetic differentiation and taxonomic level, the relative magnitude of the *D. c. coronata* vs. *D. c. auduboni* distance nevertheless does suggest that any major genetic events that may occur in the process of speciation have not occurred in this case. Electrophoretic studies of additional closely related pairs of birds will be required, however, before the extent of structural genetic changes occurring during avian speciation is known with confidence.

The small genetic distance between these warbler taxa is reflected by the fact that the among-population component of genetic variance, $\hat{F}_{st}$, is very small and not significantly different from zero (Table 4). This is in conformance with the general trend of $\hat{F}_{st}$ values for birds reported in Table 5. Only the regional component of genetic variance between the Rocky Mountain and the California coastal subspecies of *Zonotrichia leucophrys* was found to be significantly greater than zero in my analysis. These regions are separated by a large expanse of uninhabited terrain, and this, among other factors such as selection, might result in such a genetic difference. The value for *Aplonis cantoroides* is sufficiently large to suggest the existence of population subdivision in that species, but the result is based on only two variable loci and is associated with a large standard error. Thus, it would appear that a small fraction of the total genetic variance is distributed among continuously distributed populations of birds, at least for the species examined to date. This may be due to the relatively large amount of gene flow and the moderate to large effective population sizes found in the species of birds for which estimates are available (Barrowclough 1980).

Phenotypic differentiation.—Plumage patterns are the product of regulatory genes. Hence, the analysis of such characters offers the potential to examine the extent of evolution of nonstructural genes (King and Wilson 1975, Wilson 1975). The hybrid index scores from the birds collected in association with this study are in agreement with Hubbard's (1967) findings. There has not been any major change in the position or shape of the cline in the decade since Hubbard's study, and the major transition from the *D. c. coronata* phenotype to the *D. c. auduboni* phenotype still occurs over a distance of less than 200 km. Although there is sufficiently strong phenotypic differentiation in this complex that the two forms were originally thought to be separate species, the preceding analysis indicates that the genetic differentiation typical of species of birds examined to date does not exist. Also, as Hubbard (1967: 128) pointed out, behavioral isolation, in the form of assortative mating in the zone of hybridization, does not appear to be present.

Interpretation.—Several authors have attempted to reconstruct the events leading to the differentiation of these two taxa. Geographical isolation of the eastern boreal forest with the *D. c. coronata* form from the western cordilleran forest with the *D. c. auduboni* form was supposed to have occurred during the Wisconsin glaciation. This may have been mediated by such conditions as an unforested area in the Great Plains (Mengel 1964), extensive glaciers and alpine tundra along the backbone of the Rocky Mountains (Hubbard 1967, 1969), or blowing dust or sand (Mengel 1970). The first two phenomena, however, are at variance with the presently available palynological data for the last 20,000 yr, and the magnitude of the last phenomenon is unknown due to a lack of paleoecological sites from the critical areas of the northern Great Plains (Wright 1971, Ritchie 1976). Nevertheless, it does appear as though much of the northern Great Plains was occupied by spruce forest during the last glaciation. Thus, the evidence for an extended period of geographical isolation of these two taxa in the late Wisconsin or earlier is circumstantial. The possibility of differentiation during allopatry does remain, however, because these vegetational reconstructions of western North America are based on few data, and refugia may yet be found. Because of this lack of direct evidence for differentiation during allopatry, other modes of differentiation ought to be considered in view of the current pluralism in thought concerning speciation mechanisms. Bush (1975), Endler (1977), and White (1978a) recently reviewed the hypothesized modes of speciation, and of the various modes two, clinal and stasipatric effects, are at least plausible mechanisms in this case and will be discussed below in addition to allopatry.

If it is assumed that *D. c. coronata* and *D. c. auduboni* are in secondary contact following divergence during geographical isolation, then it is of interest to estimate the time at which this contact first occurred. It appears that Pine Pass and other such areas of the Canadian Rockies became ice free approximately 10,000–12,000 yr ago (Prest 1970, Luckman and Osborn 1979), but there is no direct evidence as to when the pass was reforested. Hubbard (1967) estimated that this occurred at least 4,000 yr ago. A better, but still speculative, estimate of the time of reforestation with the appropriate vegetation for Yellow-rumped Warblers (spruce forests) can be obtained by noting that, for a well-studied site northwest of Pine Pass in the Yukon, glaciation receded about 12,500–13,500 yr ago, and reforestation, based on a pollen profile, occurred about 8,500–9,000 yr ago, or approximately 3,500–5,000 years after deglaciation (Prest 1970, Rampton 1971). At a second site southeast of the Pine Pass area in Jasper National Park, reforestation occurred between 8,000 and 9,000 yr ago, about 2,000–3,000 yr after deglaciation (Schweger 1978). These two localities for which data are available bracket the transect studied; if the relative timing of events was similar at Pine Pass, then contact of the warblers (assuming they reoccupied forests without much delay) would have occurred between 2,000 and 5,000 yr after the recession of ice, or 5,000–10,000 yr ago. In order to get a point estimate for illustrative purposes, I will assume contact occurred midway through this interval, or 7,500 yr ago.

The current state of the hybrid zone has been described and some information concerning its history estimated. To understand the current status, however, it is necessary to try to infer the magnitudes of the evolutionary forces at work in the zone. My results indicate that the widths of the overall phenotypic cline and the individual character clines have not changed detectably in the last decade. Hence, the width of these clines, measured as the distance between the points where the populations take on average phenotypic values of 20% and 80% of the "pure" values far from the contact zone, is approximately 150 km. A second parameter that is necessary for further analysis of the history and current status of these populations is gene flow. Barrowclough (1980) has presented data suggesting that the magnitude of gene flow in passerine birds is typically of the order of 10^3 m/yr. Because that result included the effects of overlapping generations, this number also is the amount of gene flow per generation.

It is possible to analyze the forces acting on these warblers using several different classes of models of clines, given the above estimates of some of the requisite parameters. I will treat the plumage differences between the two phenotypes as though each character were determined by a single locus; this assumption will be discussed later.

The first possibility is that of a dynamic cline. That is, suppose the two taxa did come into secondary contact 7,500 yr ago and that the plumage differences between them are selectively neutral. Then there would be introgression of the traits of each taxon into the range of the other at a rate determined only by gene flow. For such a case, what would the situation be like today? Endler (1977) reports that single locus simulations for such situations, assuming large populations and homogeneous gene flow, suggest that the width of the cline, as measured above, increases according to the relationship $w \simeq 1.68 l \sqrt{T}$, where w is the cline width, l is the root-mean-square gene flow, and T is the time since secondary contact. For the values arrived at above, this yields an estimate of 145.5 km; as noted above, the actual cline width is approximately 147.3 km. While the agreement of the prediction with the independently observed width is remarkable, it must be remembered that there are many estimates involved in the calculation of the expected width. The conclusion warranted is merely that the prediction is of the same order of magnitude as the observed width.

An alternative procedure is to assume the width of 147.3 km and estimate the age, using a transformation of the simulation equation, to see if the age obtained is in agreement with the known geological history of the area. The result obtained, 7,687 yr, is in reasonable agreement with this history.

There are several static cline models involving selection-gene flow equilibria that also might apply. It is possible that the transition between the two forms occurs at an ecotone, that is, the environment changes in the region of contact so that traits of the *D. c. coronata* phenotype are favored on one side and those of the *D. c. auduboni* phenotype on the other. Slatkin (1973), May et al. (1975), and Nagylaki (1975) have shown that, for no dominance, large populations, and homogeneous gene flow, such single-locus clines scale as a function of $l_c = l/\sqrt{s}$, where s is the coefficient of selection acting on the trait. In general, if Δ is the distance over which the environment changes from favoring one morph to the other, then $w \simeq 2.08l_c$ for $l_c > \Delta$, and $w \simeq 1.66(l^2_c\Delta)^{1/3}$ for $l_c < \Delta$ (May et al. 1975). In Endler's (1977) book, these are called the ecotone and gradient models, respectively. For this case, estimates are available for all parameters except s, which can therefore be estimated. If it is assumed that the environmental transition is extremely sharp and occurs over a few kilometers at the top of Pine Pass, then $\hat{s} \simeq 1.9 \cdot 10^{-4}$. If it is assumed that the critical environmental gradient is the change from the white spruce forest of the north and east to the Engelmann spruce forest of the west, then the transition occurs over approximately 64 km (Chapman et al. 1956). For this case Δ again is found to be less than l_c, and the estimate of s remains $1.9 \cdot 10^{-4}$.

While these estimates of the width of a potentially critical environmental gradient seem reasonable to me, it might be argued that the important change actually occurs over a much greater distance. For such a case Δ is greater than l_c, and larger estimates of the selection coefficient, s, can be obtained. This critical width has to be quite large, however, to get values of s that are at all appreciable. For example, $\hat{s}$ is 10^{-3} when Δ is 700 km, and $\hat{s}$ only becomes 10^{-2} if Δ is of the order of 7,000 km.

These results are essentially invariant to any level of simple dominance (May et al. 1975, Endler 1977). Heterozygote advantage or disadvantage, however, is difficult to take into account. As Moore (1977) pointed out, heterozygote superiority can account for a stable hybrid zone of any shape and size; this is a very powerful yet hard-to-dissect phenomenon. A small amount of heterozygote disadvantage only slightly steepens a cline. When the shape of a cline is determined predominantly by heterozygote disadvantage (i.e. $|h| \gg s$, where $|h|$ is the magnitude of the fitness disadvantage of heterozygotes), however, the cline will scale as $l/\sqrt{|h|}$ (May et al. 1975). If this is used to estimate $|h|$, then a value on the order of $4 \cdot 10^{-5}$ is obtained. Heterozygote disadvantage is the kind of effect one might expect if the gene pools of the two forms had differentiated in allopatry, different alleles had become fixed, and on contact these alternate alleles proved incompatible; hybrid sterility, for instance, is an example of this. The results reported above demonstrate that such an effect need only be of a small magnitude to produce the existing pattern.

For the heterozygote advantage model, the width of the cline varies as a function of the quotient h/s (Slatkin 1973, May et al. 1975). Consequently, there is an infinite set of h, s values consistent with the cline. For ratios of h to s that seem reasonable ($h < s$), $\hat{s}$ is of the order of 10^{-4} and smaller for the ecotone model. For the gradient model, no pair of values of h and s for which $2h$ is less than s can be easily ruled out. For values of $\hat{s}$ as large as 10^{-2}, however, it appears as though Δ must be of the order of 10^4 km or more (May et al. 1975: Fig. 6). Heterozygote advantage is expected in this case if alleles fixed in the two "pure morph" ranges also happen to be overdominant only in the narrow hybrid area. This might be expected, for example, if the area itself is intermediate for some important factor.

Wright (1931) suggested that the relative efficacy of selective and random genetic processes in populations is determined by the relative magnitudes of effective population size, N_e, and $1/(4s)$. The above estimates suggest that for this hybrid zone, s may be of the order of 10^{-4}; hence, $1/(4s)$ is approximately 2,500. Barrowclough (1980) estimated that the species of passerines for which there are data have values of N_e of the order of 10^2–10^3. Thus, it appears that for this case selection may be relatively small and of the same order of magnitude as random effects. Nagylaki (1978) provides a method for checking this conclusion. He has shown that if β, equal to $2\rho l^2/l_c$, is much greater than 1, then selection is highly significant in a cline. If β is much less than 1, then random genetic drift is important. Estimates of l and s are available from the above analysis; the density of birds, ρ, was estimated as 49.2/km^2 based on my field observations of 20 territories along a 4,023 m transect near the McLeod Lake study site. These estimates result in an estimate of β of 0.98. This is consistent with the above conclusion that natural selection and random effects are of the same order of magnitude in this cline.

Both the analysis of the expected width of a cline given complete neutrality and the estimate of the order of magnitude of selection coefficients suggest that one need not postulate large selective pressures maintaining the differences between the two races of this warbler. This inference is based on the use of single-locus models. The fact that several different phenotypes can be distinguished within populations in the hybrid zone, however, suggests that the phenotypic traits are probably the product of a few genes (Falconer 1960: 105–106). Multi-locus models of selection-gene flow clines have not been used in this analysis because they involve extremely complicated algebra and consequently are not well developed. Slatkin (1978), however, has shown that approximately the same general classes of results obtain for geographically variable, additive, polygenic traits. Further, he has demonstrated for a two-locus case that, unless the loci are tightly linked, linkage disequilibrium will be small, and single-locus results will obtain (Slatkin 1975: 792). For passerine birds, with diploid numbers in the 70's and 80's, close linkage, although possible, seems improbable. For the case of a dynamic cline, the justification for the use of a single-locus model appears to be even greater. In the absence of selection, multiple loci should act to make the results more deterministic by reducing, at the phenotypic level, the effects of sampling error associated with gene flow. Consequently, the use of single-locus models appears to be justified when selection is small or absent. Thus, the results of this analysis do not violate the assumptions. The behavior of polygenic models under strong selection are unknown; hence, while such a situation cannot be ruled out, it does not seem parsimonious to invoke such an unknown phenomenon.

The question of the robustness of the estimates needs to be addressed also. The above analysis suggests that the estimates of the width of the cline and the elapsed time since the reforestation of the pass probably are accurate to within 25%. The estimate of gene flow (l) though, is based on studies of several other passerines and could be too large or small by a factor of two. I have obtained estimates of s using these extreme values of gene flow; these analyses suggest that s is probably between $4 \cdot 10^{-5}$ and $8 \cdot 10^{-4}$. For these cases, β remains on the order of 1.

Given these estimated magnitudes of evolutionary forces in the region of intergradation, the question of the history of the complex needs to be addressed. Currently there is controversy over the interpretation of steep clines between well-differentiated taxa. One school holds that such cases represent populations differentiated during allopatry and arrested by secondary contact (Mayr 1963, 1970). A second maintains that such differences may represent primary intergradation due to either a stasipatric chromosomal rearrangement or disruptive selection due to strong gradient effects (Endler 1977, White 1978a). If the selective forces at work in this cline are actually of the magnitude estimated, then this would seem to constitute circumstantial evidence against a stasipatric origin of the two taxa. In White's (1978a,b) model, chromosomal variants are essentially fixed parapatrically with F1's at a large disadvantage due to reduced fecundity or viability. This leaves allopatric and gradient effects as potential causes for the differentiation of the two forms.

It is exceedingly difficult to distinguish between secondary and primary contact (Endler 1977). Because the vegetational history is uncertain and there is no electrophoretic evidence for a genetic revolution, the case for allopatry is not overwhelming. The case for primary intergradation, however, is also weak. The estimates of selective differentials are very small, and the current width is completely consistent with the contact of neutral, alternatively fixed alleles 7,500 yr ago. It is not obvious why several characters should all have combinations of dominance and selective regimes so as to yield nearly identical cline widths. In addition, a number of other taxa (birds, butterflies, and trees; Remington 1968) also meet in the same place; while not unambiguous, all this is suggestive of expansion from refugia and hence of an allopatric origin (Jackson and Pounds 1979). Thus, the evidence may be construed to be somewhat in favor of an allopatric origin, but a more definitive answer will probably have to await better paleoecological data on the existence and location of Pleistocene refugia in western North America and their subsequent history during expansion.

Taxonomy.—In general, taxonomic judgements may be easier to make once the evolutionary forces involved in a complicated situation are worked out. For instance, in this case it has been shown that the two taxa are very similar and comparable to other avian subspecies with respect to the structural genes sampled. If the phenotypic cline is dynamic, then it is consistent with the diffusion of selectively neutral alleles following secondary contact. If in equilibrium, the selective forces may be very small and of the same magnitude as random effects. Hence, any alleles for positive assortative mating that currently segregate, or that subsequently arise, will have little chance of spreading deterministically. Thus, in terms of potential outcome given the current status, there is no more reason to treat these two taxa as species than there is for any other case of geographical variation in plumage characters. The forms easily satisfy the 75% rule (Mayr 1969), however, and so are best treated taxonomically as subspecies, *Dendroica c. coronata* and *D. c. auduboni*.

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