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GENETIC DIFFERENTIATION, GEOGRAPHIC VARIATION AND HYBRIDIZATION IN GULLS OF THE *LARUS GLAUCESCENS-OCCIDENTALIS* COMPLEX[1]

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Abstract. Survey of morphometric, colorimetric, and allozymic variation in the Glaucous-winged Gull (*Larus glaucescens*), the Western Gull (*L. occidentalis*) and their hybrids is based on 706 specimens taken from 33 colony areas located throughout the breeding range of both species. Whereas most morphometric characters overlap between taxa, colorimetric characters exhibited significant intraspecific and interspecific clinal variation. Canonical discriminant function analysis of colorimetric characters clearly separated pure and hybrid morphotypes and confirmed the intermediacy of character expression in hybrids. Plumage melanism of the mantle and primary tips are among the best discriminators of hybrids. Twenty-five of 32 presumptive genetic loci were polymorphic. Although there were no fixed differences between taxa, gene frequencies exhibited significant heterogeneity across geography. Despite moderately high gene flow ($Nm = 2.8$ individuals per generation), populations of the *L. glaucescens-occidentalis* complex are not panmictic, and the hybrid zone forms a partial barrier to gene flow. Hybrids are genetically more similar to *L. glaucescens* than to *L. occidentalis*. The hybrid zone extends from Juan de Fuca Strait, Washington, south to Coos Bay, Oregon. A broad zone of introgression extends from the Queen Charlotte Islands in British Columbia south to Coos Bay, Oregon. The hybrid zone appears to have expanded in recent years, yet its midpoint near Grays Harbor, Washington, has remained stable. Hybridization appears to be responsible for skewed introgression and elevated genic diversity in *L. glaucescens*. The fact that *L. glaucescens* shows high levels of introgression while maintaining its genetic identity lends support to the hypothesis that hybridization in birds may serve as a mechanism for increasing or restoring genetic diversity in forms that are adapted to local environments.

Key words: allozymes; genetic differentiation; gene flow; geographic variation; gulls; hybridization; Larus; *morphometrics.*

INTRODUCTION

A central tenet of evolutionary biology is the origin of species through geographic subdivision of populations (Mayr 1963). Subdivision of populations into disjunct units spread out over great distances is thought to permit local differentiation via stochastic and deterministic processes that may lead to reproductive isolation and speciation (Endler 1977). When contact between divergent populations is reestablished, hybrid zones can form with resultant consequences for the evolutionary trajectories of the interbreeding populations (Barton and Hewitt 1989, Arnold 1993, Harrison 1993). For instance, if hybrid fitness is high, introgression can be extensive and the hybridizing populations may become panmictic over time, resulting in the formation of a hybrid swarm in place of the original taxa. Recent work in avian systems has shown that hybridizing taxa may experience bouts of gene exchange without becoming panmictic, especially if interbreeding is restricted in geographical or chronological scope (e.g., Parsons et al. 1993). Under such circumstances hybrid zones may function as conduits for gene exchange between species and thereby serve to increase overall levels of genetic and phenotypic diversity (Grant and Grant 1992, 1994, Moore and Price 1993, Parsons et al. 1993, Pierotti and Annett 1993). Clearly, such evidence has far reaching implications for biological species definitions because it suggests that the genetic systems of vertebrates may be prone to more introgression and lateral gene transfer than previously thought.

Populations of large, white-headed *Larus* gulls provide an excellent system for the study of differentiation and introgression because hybrid-

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ization occurs frequently within the genus (see Pierotti 1987). However, aside from the recent work of Snell (1991a,1991b), nearly all previous gull hybrid zone research suffers from two major shortcomings: (1) limited character suites are used to define hybrids, and (2) intraspecific variation in the parental species is not adequately assessed, usually due to limited geographic sampling. Such limitations affect the precision with which a hybrid zone can be characterized and identified (Schueler and Rising 1976). To fully assess patterns of variation between avian populations it is imperative to use multiple sets of characters (Zink and Remsen 1986) and, in the case of a hybrid zone, to expand sampling to include allopatric populations as well. Only in this way can the effects of introgressive hybridization be distinguished from those of intraspecific variation (Endler 1977), a prerequisite to clarifying geographic differentiation and gene flow across a hybrid zone (Barton and Hewitt 1989, Harrison 1993).

This paper presents a multidimensional approach to the study of hybridization between the Glaucous-winged Gull (*Larus glaucescens*) and the Western Gull (*Larus occidentalis*). Geographic variation in colorimetric (plumage and soft-part colors) and morphometric characters is assessed by univariate and multivariate statistical methods. Protein electrophoresis is used to investigate population genetic structure and gene flow within the complex. The use of external characters to identify hybrids is tested and, following classification by canonical discriminant function analysis, interspecific genetic differentiation is assessed. Results from these analyses are used to infer the position of the hybrid zone and the evolutionary history of the *Larus glaucescens-occidentalis* complex.

The breeding distribution of *Larus glaucescens* extends from the east coast of the Kamchatka Peninsula across the Aleutian chain and the Gulf of Alaska south through coastal British Columbia and Washington to northwestern Oregon (AOU 1983). *Larus occidentalis* breeds from Juan de Fuca Strait and the outer Washington coast south through Oregon, California and the west coast of Baja California (AOU 1983). The two species hybridize where their breeding ranges overlap along the Washington and northern Oregon coasts (Scott 1971, Hoffman et al. 1978). In addition, *L. glaucescens* hybridizes with the Slaty-backed Gull (*L. schistasagus*) on the Korjak shore of Kamchatka (Firsova and Levada 1982) and the Herring Gull (*L. argentatus*) in southern and southeastern Alaska (Williamson and Peyton 1963, Patten and Weisbrod 1974, Patten 1980). *Larus glaucescens* is also thought to hybridize with the Glaucous Gull (*L. hyperboreus*) along the eastern Bering Sea coast of Alaska (Strang 1977). Patten (1980) refers to *L. glaucescens* as a "key" taxon because it potentially links several of the northern Pacific Rim gulls.

MATERIALS AND METHODS

SAMPLING DESIGN AND ANALYSIS

Field work was carried out from 1985 to 1990 during the breeding season in coastal Alaska, British Columbia, Washington, Oregon, California, and Baja California. Thirty-three "colony areas," each including one or more collecting localities, was established throughout the allopatric and sympatric breeding ranges of *L. glaucescens* and *L. occidentalis* (Table 1, Fig. 1). Gulls were collected at nests or from sea or land points near colonies. Freshly collected birds were classified *a priori* in the field as either pure or hybrid based on general appearance in plumage and soft-part coloration (see Hoffman et al. 1978). A total of 704 specimens of *L. glaucescens, L. occidentalis,* and their hybrids was collected, and an additional 58 specimens (wing preparations) were received on loan from the Burke Museum at the University of Washington.

As a null hypothesis, all members of the *Larus glaucescens-occidentalis* complex were assumed to be invariant throughout their breeding range. Demonstration of significant geographic variation would lead to the rejection of the null hypothesis. In addition, the data were first analyzed under the assumption that each colony area was panmictic. Thus, no assumptions about taxonomic affinities were made, all specimens within each colony area were pooled, and the colony areas were analyzed population-by-population. This approach facilitated the testing of hypotheses concerned with overall variation in the complex, the shape of character clines, and Hardy-Weinberg equilibria within populations. Discriminant function analysis was then used to classify specimens to taxon (= morphotype) *a posteriori.* Each class of *a posteriori*-designated morphotype was then subjected to further analyses on intraspecific and interspecific levels.

TABLE 1. Locations of the 33 colony areas where specimens of the *L. glaucescens-occidentalis* complex were collected. Except for *L. o. wymani*, sample sizes (*n*) refer to specimens identified *a posteriori*. Alphanumeric codes correspond to numbered localities in Figure 1.

Alpha-numeric code	Colony area	*L. glaucescens* *n*	Hybrids *n*	*L. o. occidentalis* *n*	*L. o. wymani* *n*
BULD1	Buldir I., Aleutian Is., AK	19			
KACH2	Kachemak Bay, AK	8			
MIDD3	Middleton I., AK	20			
MASS4	Masset, Queen Charlotte Is., BC	13	2		
CUMS5	Cumshewa, Queen Charlotte Is., BC	29	4		
STJA6	St. James I., Queen Charlotte Is., BC	14	1		
NVAN7	Northern Vancouver I., BC	9	5		
GEOR8	Georgia Strait, BC	15	6		
CLEL9	Cleland I., BC	14	13		
SJI10	San Juan Is., WA	23	17		
TAT11	Tatoosh I., WA	14	26	1	
PRO12	Protection I., WA	8	8	1	
DES13	Destruction I., WA		13	5	
GRA14	Grays Harbor, WA	6	24	16	
PUG15	Puget Sound, WA	27	17		
WIL16	Willapa Bay, WA	2	11	4	
ESI17	East Sand Island, OR	3	21	3	
TIL18	Tillamook, OR		2	12	
YAQ19	Yaquina Bay, OR	2	8	8	
COO20	Coos Bay, OR	2	3	25	
CCY21	Crescent City, CA			16	
TRN22	Trinidad, CA			16	
SUG23	Cape Mendocino, CA			19	
MEN24	Mendocino, CA			16	
PTR25	Point Reyes, CA			14	
FAR26	Farallon Is., CA			27	
SFB27	San Francisco Bay, CA			23	
ANO28	Año Nuevo I., CA			6	
MOS29	Moss Landing, CA				11
MOR30	Morro Bay, CA				13
ANA31	Anacapa I., CA				32
SNC32	San Nicolas I., CA				19
LCO33	Los Coronados I., MX				10
	Total	228	181	212	85

MORPHOMETRIC AND COLORIMETRIC ANALYSIS

Mensural characters. The following eleven measurements were taken from freshly collected specimens (see Baldwin et al. 1931): (1) WIL = wing length, (2) TAIL = tail length, (3) TAR = length of tarsus, (4) TOM = length of middle toe, (5) HEAD = head length, (6) CUL = culmen length, (7) BLPN = Bill length, taken from the posterior border of the nare to the tip of the bill, (8) BIW = Bill width, the line across the upper mandible from one tomial ridge to the other, taken at the level of the posterior border of the nares, (9) BID = depth of the bill at the angle of the gonys, (10) BIDA = depth of the bill at the level of the anterior border of the nares, (11) BIDP = depth of the bill at the level of the posterior border of the nares.

Color characters. Mantle (MANT) and primary tip (TIP) melanism were measured on wing preparations and study skins with a Munsell 37-step neutral value scale (Munsell 1971) under a Macbeth Super Color Matching Skylight (Model BX 848A) approximating natural, north sky daylight. Colors of the bill at the nares, each eye-ring (= orbital ring), the iris, and the dark pigment of the iris, if present, were measured with hand-held Munsell color charts (matte finish) from the Munsell Book of Color (Munsell 1976). In addition, the proportion of the iris that was

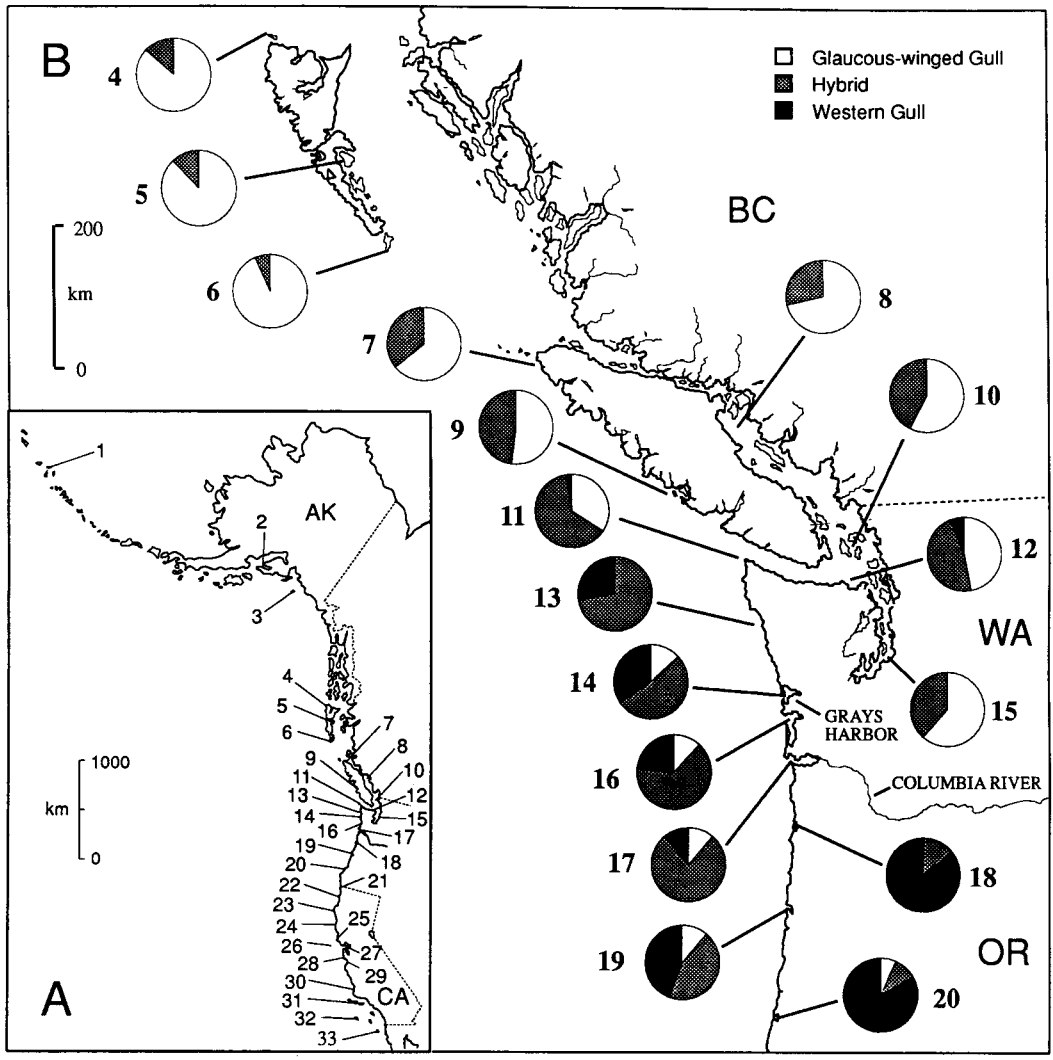

FIGURE 1. A. Map of 33 colony areas sampled in this study. Numbers correspond to colony alphanumeric codes in Table 1. B. Map of the hybrid and introgressive zones in the *L. glaucescens-occidentalis* complex. Pie diagrams illustrate the relative proportions of each morphotype collected at the given colony area. Morphotypes classified *a posteriori* by DFA. Numbers next to each pie diagram correspond to colony alphanumeric codes (Table 1). The hybrid zone is defined here as the region of breeding sympatry between pure *L. glaucescens* and *L. o. occidentalis,* and encompasses colonies 12 (PRO12) to 20 (COO20), excluding 15 (PUG15). Its midpoint is centered around colony 14 (GRA14). The zone of introgression is the region where hybrids were collected as breeding birds, and encompasses colonies 4 (MASS4) to 20.

covered by dark pigment (e.g., 25%, 50%, 75%, 100%) was noted. Because soft part colors fade soon after a specimen is collected, color comparisons had to be made under ambient lighting conditions in the field. Color charts were the only practical method available for quickly measuring the small and irregular surface areas of gull eyelids and irides in the field.

Each Munsell color is defined by three characters—hue, value, and chroma. From these, dominant wavelength (DW), percent spectral reflectance or brightness (Y), and excitation purity (PE), respectively, can be obtained with the aid of conversion tables (Munsell 1968). Newhall et al. (1943) and Bowers (1956) discuss the basis for these conversions. For examples on the use

of Munsell color notations in avian systematic studies, see Hellack and Schnell (1977) and Smith (1987). Endler (1990) discusses the limitations inherent in color determinations based on visual inspection of color chip standards. Abbreviations for the soft-part colorimetric characters analyzed here are: PIPO = proportion of iris covered in dark pigment; BIY = bill color, brightness; BDW = bill color, dominant wavelength; BPE = bill color, excitation purity; EY = eye-ring, brightness; EDW = eye-ring, dominant wavelength; EPE = eye-ring, excitation purity; E2Y = second eye-ring, brightness; E2DW = second eye-ring, dominant wavelength; E2PE = second eye-ring, excitation purity; IY = iris, brightness; IDW = iris, dominant wavelength; IPE = iris, excitation purity; PY = iris pigment, brightness; PDW = iris pigment, dominant wavelength; PPE = iris pigment, excitation purity.

Numerical analysis. Colorimetric variables were analyzed separately from morphometric data (see Burtt 1986). Specimens with missing data were excluded from a given analysis. Most statistical routines were run on SAS (SAS Institute 1990). All character distributions did not deviate significantly from normality (SAS-UNIVARIATE). Multivariate analysis of variance was used to test for effects of sex and taxon differences (MANOVA, SAS-GLM). Because of significant sexual dimorphism, all mensural characters and some colorimetric characters were separated by sex for analysis (see below). Population means were compared with the sums of squares simultaneous test procedure (SS-STP; Gabriel and Sokal 1969) using a program written by Sokal and Rohlf (1981) as modified by Krogman and Stangenberger.

Prior to running statistical routines that are affected by scaling (see Sneath and Sokal 1973), mensural characters were log-transformed (Jolicoeur 1963) and colorimetric characters were standardized. Principal components analysis (PCA) of the variance-covariance matrix of 11 log-transformed mensural characters was used to summarize variation across all characters (SAS-PRINCOMP).

Canonical discriminant function analysis (DFA) of colorimetric characters was employed to classify specimens into three groups separated by sex: putative *L. glaucescens, L. o. occidentalis,* and hybrids. The southern Western Gull, *L. o. wymani* (MOS29-LCO33), was not included in the DFA. To calibrate the DFA, specimens collected from largely allopatric colonies were designated pure. Thus, specimens from BULD1 to STJA6 (see map, Fig. 1) were used to represent *L. glaucescens,* while those collected from colonies COO20 to ANO28 represented *L. o. occidentalis.* Specimens collected from colony areas NVAN7 to YAQ19 were assigned to the hybrid calibration data set after removal of specimens considered to be putatively pure, based on *a priori* assumptions of plumage and soft-part color variation. To obtain *a posteriori* classifications, all specimens from colonies BULD1 through ANO28, separated by sex, were then subjected to linear DFA (SAS-DISCRIM) using the pooled covariance matrix of Euclidean distance values calculated from the two plumage and 16 soft-part colorimetric characters described above. Calculating the *a posteriori* error rate probability permitted the classification of specimens of questionable pure or hybrid status.

ALLOZYME ELECTROPHORESIS

Sample preparation and laboratory procedures. Tissue samples of liver, heart, kidney and pectoral muscle were removed from specimens in the field and frozen in liquid nitrogen. The Aleutian Island (BULD1) and the Farallon Island (FAR26) specimens were frozen whole at −20°C and shipped frozen to Berkeley, California. Tissue homogenates were prepared as outlined in Johnson et al. (1984) and all tissue extracts were stored at −70°C. Protein separation by standard horizontal starch-gel electrophoresis (Selander et al. 1971, Yang and Patton 1981) was optimized using Hackett's (1989) sequential electrophoretic method. Proteins were assayed according to the methods of Harris and Hopkinson (1976) and Selander et al. (1971). Twenty-five enzyme systems representing 33 presumptive genetic loci were assayed (Appendix 1). Alleles for each locus were scored alphabetically in order of decreasing mobility. One locus, PNP, was eliminated from the analysis because of inconsistent scoring.

Numerical analysis. Unless otherwise noted, all standard genetic variability measures, distances and clustering procedures based on electrophoretic data were computed with the program BIOSYS-1 (Swofford and Selander 1981). Percent polymorphism (*P*) was calculated using the 0.99 criterion. Average heterozygosities (*H*) were calculated by direct count. Variable loci in each population were tested for departure from Hardy-Weinberg equilibrium with the chi-square

goodness-of-fit test using Levene's (1949) correction for small sample sizes and with an exact significance probability test (Elston and Forthofer 1977). The significance level in the chi-square tests, 0.05, was divided by the number of simultaneous tests for each locus, in this case, 32, to yield a modified significance level of 0.0015. This procedure accounts for deviations from Hardy-Weinberg expected by chance alone (Cooper 1968). The fixation index (F), as defined in Hedrick (1983), was used to examine heterozygote deficiency or excess.

Genetic substructuring of populations was investigated using F-statistics (Wright 1978, Nei 1977) and principal components analysis (Barrowclough and Johnson 1986). In the latter analysis, allellic frequencies of seven of the "most variable" loci (EST-2^{a,b,c}, EAP-1^{a}, LA^{a}, PAP^{a,b}, MDPH^{a,b}, G3PDH^{a,b}, PGM-1^{a}) at all 33 colonies were arcsine transformed before subjecting the resulting variance-covariance matrix to PCA. The genetic distance measures of Nei (1978) and Rogers (1972) were used to assess divergence between colony areas and taxa. Phenetic clustering of taxa based on genetic distance was accomplished using the unweighted pair-group method with arithmetic averaging (UPGMA; see Sneath and Sokal 1973).

Quantitative estimates of gene flow were obtained using F_{ST}, a measure of intercolony variation in allele frequency averaged across all alleles, loci and colonies, to estimate Nm from the formula developed by Wright (1951): $Nm = (1/F_{ST} - 1)/4$, where N is the number of individuals in a population and m is their migration rate (individuals per generation). This formula assumes an infinite island model of population structure at equilibrium and neutral allelic variation (Wright 1951).

MORPHOMETRIC AND COLORIMETRIC RESULTS

MORPHOMETRIC VARIATION

MANOVA revealed that all 11 mensural characters in the *L. glaucescens-occidentalis* complex are significantly sexually dimorphic, with males being on average 6–13% larger than females (Bell 1992). Mensural variation was therefore analyzed separately by sex. The 11 log-transformed mensural characters loaded positively on PC1 and variably on PC2 for each sex in the PCA (Table 2). This pattern of character loading implies that PC1 scores summarize mostly size-related variation, whereas PC2 scores summarize both size and shape variation (Marcus 1990). In both sexes, the total variance explained in the PCA is greater for PC1 than PC2 (Table 2). Plots of PC1 versus PC2 showed extensive overlap between morphotypes in the complex (Bell 1992).

TABLE 2. Loadings on first two principal components (PC) for mensural characters. Morphotypes pooled, sexes analyzed separately. Character abbreviations explained in Methods.

Variable	Males		Females	
	PC1	PC2	PC1	PC2
	$n = 318$		$n = 271$	
log WIL	0.57	0.33	0.35	0.35
log TAIL	0.56	0.50	0.48	0.59
log TAR	0.61	−0.02	0.54	−0.02
log TOM	0.46	−0.25	0.43	−0.25
log HEAD	0.78	0.38	0.72	0.40
log CUL	0.71	0.51	0.65	0.50
log BLPN	0.69	0.52	0.66	0.59
log BIW	0.68	−0.45	0.68	−0.26
log BID	0.76	−0.23	0.74	−0.28
log BIDA	0.79	−0.31	0.83	−0.29
log BIDP	0.75	−0.38	0.77	−0.37
Eigenvalues[1]	0.22	0.08	0.22	0.08
Proportion of variance	0.465	0.163	0.439	0.153

[1] $\times 10^{-2}$.

The SS-STP identified eight subsets of maximally non-significant means for male PC1 scores (pooled taxa), showing that although this multivariate measure of size varies within the complex, no pattern of significant clinal variation is evident due to extensive overlap of colony means (Fig. 2). Within the complex, however, males from the Aleutians Islands (BULD1) tend to be the largest, whereas those from the Channel Islands off southern California and Baja California (ANA31 to LCO33) tend to be the smallest. Females exhibit a smiliar trend in PC1 scores (Bell 1992). Univariate mensural characters, such as head, wing, and tarsus length, revealed similar patterns of variation in size in both sexes (Bell 1992). Extensive overlap between morphotypes of the *L. glaucescens-occidentalis* complex in British Columbia, Washington and Oregon, precluded using morphometric characters to discriminate hybrids from pure parentals.

COLORIMETRIC VARIATION

Results from the MANOVA revealed that one or more characters related to either bill, eye-ring,

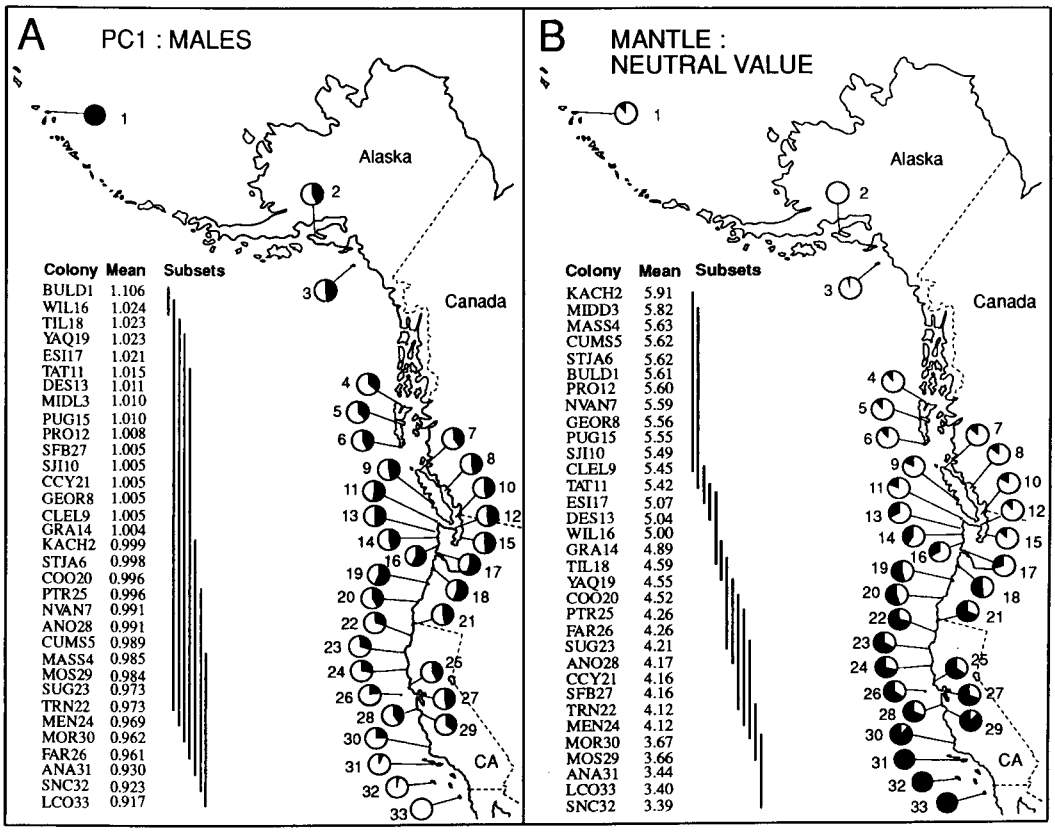

FIGURE 2. A. SS-STP applied to colony area means for male PC1 scores in the *L. glaucescens-occidentalis* complex, morphotypes pooled. Population means are listed to the right of colony alphanumeric codes. The pie diagrams at each locality represent the mean of the character at that locality, scaled as a percentage of the total range of means across all colonies. Vertical bars represent maximally non-significant subsets of population means. B. SS-STP applied to colony area means for mantle neutral value in the *L. glaucescens-occidentalis* complex, morphotypes pooled, sexes combined. Mantle neutral value colony means ranged from dark gray (SNC32) to light gray (KACH2).

or iris color were sexually dimorphic (Bell 1992). As Pierotti (pers. comm.) pointed out, the bills of females appear "richer", less "faded" in color than those of males. Males and females did not differ significantly in their respective distributions of neutral values for the mantle (Kolmogorov-Smirnov two sample test, $D = 0.029$, $P > 0.05$) and primary tips (K-S two-sample test, $D = 0.069$, $P > 0.05$). Therefore, the sexes were combined to study geographic variation in the population means of these two characters.

Results from the SS-STP applied to colony means (pooled taxa) for mantle neutral value reveal significant clinal variation with latitude (Fig. 2), ranging from dark gray (mean = 3.39) at SNC32 in southern California to light gray (mean = 5.91) at KACH2 in Kachemak Bay, Alaska. Steps in colony means for mantle neutral value occur both within and between taxa. In *L. o. wymani*, gulls from the Channel Islands (LCO33-ANO30) have darker mantles than those from the central California coast (MOR30-MOS29). Another step in mantle neutral value occurs between *L. o. wymani* (MOS29) and *L. o. occidentalis* (ANO28). From ANO28 to CCY21, a region corresponding to pure *L. o. occidentalis*, mantle color remains fairly constant, but then increases in value, e.g., becomes lighter, in Oregon (CCO20 to TIL18), due in part to the presence of hybrids and some *L. glaucescens*, but also due to clinal variation in *L. o. occidentalis*. For example, specimens of putatively pure *L. o. oc-*

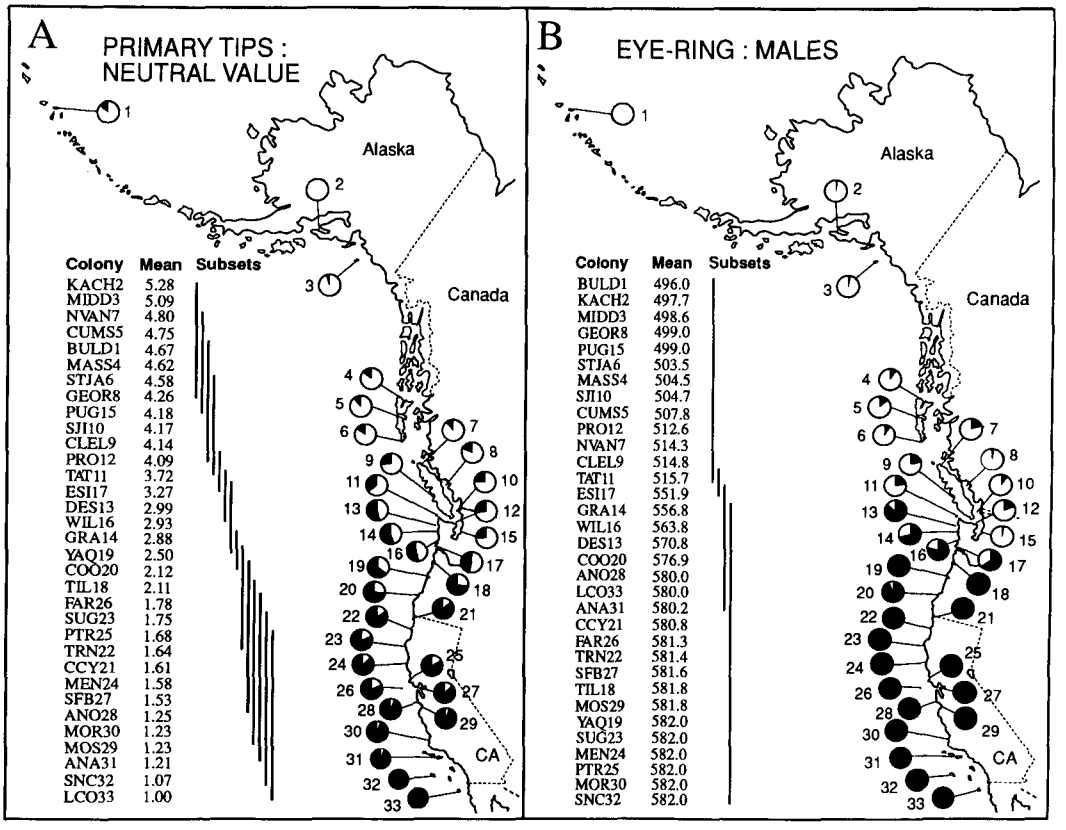

FIGURE 3. A. SS-STP applied to colony area means for primary tip neutral value in the *L. glaucescens-occidentalis* complex, morphotypes pooled, sexes combined. Primary tip neutral colony means ranged from black (LCO33) to light gray (KACH2). B. SS-STP applied to colony area means for average eye-ring dominant wavelength (DW) in males of the *L. glaucescens-occidentalis* complex, morphotypes pooled. Colony means ranged from orange-yellow (SNC32) to vinaceous-pink (BULD1).

cidentalis from Washington and Oregon have significantly lighter mantles than those from northern California (WA & OR: $MANT_{mean}$ = 4.50; n = 75; CA: $MANT_{mean}$ = 4.18; n = 133; two-tailed t_s = 11.45; $P < 0.0001$). Mantle color goes through an inflection point in colonies at the Columbia River and along the outer Washington coast (ESI17, WIL16, GRA14, DES13). These four colonies are included in the main region of sympatry. North of this zone, from TAT11 at the entrance to Juan de Fuca Strait to MASS4 in the Queen Charlotte Islands, and east into Puget Sound (PUG15), mantle color is substantially lighter, but relatively constant in mean colony value. A further step towards lighter mantle neutral values occurs between British Columbia (MASS4) and Alaska (MIDD3). Note that colony means for mantle color at STJA6 and BULD1 are nearly identical (Fig. 2). These colonies are at approximately the same latitude (52°) on opposite sides of the Pacific. Thus, *Larus glaucescens* may exhibit parallel latitudinal clines in mantle color along both eastern and western coasts of the northern Pacific Rim, although in the eastern Pacific introgression from *L. schistasagus* may also be influencing mantle color.

Similar to the mantle, colony means (pooled taxa) for primary tip neutral value exhibit significant clinal variation (Fig. 3), ranging from near-black (mean = 1.00) at LCO33 to light gray (mean = 5.28) at KACH2. Steps in primary tip melanism occur at approximately the same geographic positions as in the mantle, and colony mean subsets appear to reveal an inflection point concordant with the concentrated region of sympatry on the outer Washington Coast (ESI17, WIL16, GRA14, and DES13). As in the mantle, primary tip melanism shows intraspecific clinal

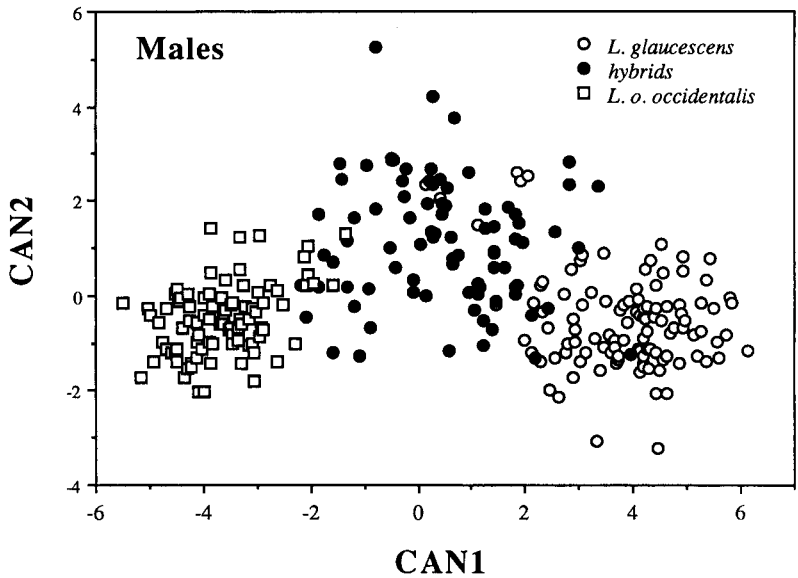

FIGURE 4. Canonical DFA of male gulls based on colorimetric variables (see Methods). Results for females were similar. Character loadings are listed in Table 3.

variation: *a posteriori*-identified specimens of *L. o. occidentalis* from Washington and Oregon have significantly lighter primary tips than those from northern California (WA & OR: $TIP_{mean} = 1.96$; $n = 75$; CA: $TIP_{mean} = 1.64$; $n = 133$; two-tailed $t_s = 10.36$; $P < 0.0001$).

The dominant wavelength (DW) of eye-ring color showed little geographic variation within putative taxa for either sex (Fig. 3). Although most gulls possessed one pigment, either yellow (DW = 575–582 nm), as in *L. occidentalis,* or pink (DW = 493–510 nm), as in *L. glaucescens,* some of the hybrids possessed both pigments (yellow and pink). It was therefore necessary to calculate an average for each gull to account for double pigments. In both sexes colony means for average eye-ring DW undergo a jump in the space of 90 km along the outer Washington coast: that is, between DES13 (47°41′N) near La Push and TAT11 (48°23′N) near Cape Flattery. Eye-ring DW demarcates a main contact boundary between *L. glaucescens* and *L. o. occidentalis.*

DISCRIMINANT FUNCTION ANALYSIS

Canonical DFA using 18 colorimetric variables provided significant discrimination of putatively pure and hybrid gulls of both sexes. Because the results are nearly identical in both sexes, only males are illustrated (Fig. 4). The first canonical variate (CAN1) separated all three categories of gulls—*L. glaucescens, L. o. occidentalis* and hybrids—and accounted for 92.8 and 93% of the within-class variance in males and females, respectively (Table 3). The second canonical variate (CAN2) provided additional discrimination of some hybrids. Hybrids are clearly intermediate in canonical multivariate space between *L. glaucescens* and *L. o. occidentalis,* although the DFA provided better separation between hybrids and *L. o. occidentalis* than between hybrids and *L. glaucescens* (Fig. 4).

Character loadings on each canonical axis signify the relative contribution of the character towards total canonical structure (Marcus 1990). Although most colorimeteric characters had high loadings on CAN1 and intermediate loadings on CAN2 (Table 3), some may serve as better discriminators than others. In both sexes, MANT and TIP load heaviest on CAN1 ($r \geq 0.90$), suggesting that these two characters contribute the most toward discriminating between *L. glaucescens, L. o. occidentalis,* and hybrids. In males, BDW also loads highly on CAN1.

THE HYBRID ZONE

Based on the *a posteriori* classification of specimens from the DFA, the hybrid zone between *Larus glaucescens* and *Larus occidentalis* is defined here to include all colonies where both species were collected as breeding birds (Fig. 1). This

TABLE 3. Loadings of colorimetric characters on canonical axes 1 and 2 from discriminant function analysis (DFA) of specimens in the *L. glaucescens-occidentalis* complex. Sexes analyzed separately. All canonical correlations significant at the $P < 0.0001$ level. Characters abbreviations explained in Methods.

Character	Males		Females	
	CAN1	CAN2	CAN1	CAN2
PIPO	0.618	0.382	0.618	0.264
MANT	0.944	0.252	0.932	0.284
TIP	0.979	0.063	0.986	0.069
BIY	0.243	0.152	0.402	−0.100
BDW	−0.958	−0.286	−0.740	−0.279
BPE	−0.131	−0.167	−0.377	0.004
EY	−0.794	0.257	−0.814	0.135
EDW	−0.838	−0.042	−0.855	−0.266
EPE	−0.803	−0.105	−0.833	−0.346
E2Y	−0.901	0.434	−0.757	0.424
E2DW	−0.842	0.308	−0.851	0.098
E2PE	−0.828	0.139	−0.855	−0.161
IY	0.152	0.023	0.241	0.106
IDW	0.477	−0.015	0.446	0.079
IPE	−0.564	−0.319	−0.523	−0.220
PY	−0.223	−0.282	−0.088	−0.194
PDW	0.209	0.164	0.404	0.284
PPE	−0.009	0.270	0.321	0.363
Canonical correlation	0.957	0.677	0.962	0.698
Proportion of variance	0.928	0.072	0.930	0.070

includes nine colony areas extending across 745 km of coastline from Protection Island (PRO12) in eastern Juan de Fuca Strait, Washington, west to Tatoosh Island (TAT11) at Cape Flattery, and then south along the coast to Coos Bay (COO20), Oregon. The zone of introgression refers to those colony areas where hybrids were collected as breeding birds, and includes all colonies in sequential order from the northern end of the Queen Charlotte Islands (MASS4), British Columbia, south to Coos Bay (COO20), Oregon. By these definitions, Puget Sound (PUG15) is excluded from the hybrid zone but included in the zone of introgression. Note that while the southern borders of the hybrid and introgressive zones coincide at Coos Bay, Oregon, the northern borders do not (Fig. 1). The hybrid zone ends at Juan de Fuca Strait, whereas the introgressive zone is continuous up to the Queen Charlotte Islands in British Columbia. Thus, the zone of introgression is skewed towards the north and east, that is, towards *L. glaucescens*. The midpoint of the hybrid zone is centered around Grays Harbor (GRA14), Washington.

Given the vagaries of small sample sizes (Table 1), there are differences in the relative proportion of morphotypes at colonies within the hybrid and introgressive zones (Fig. 1). In the hybrid zone, for example, colony composition ranges from 7% to 47% *L. glaucescens*, 10% to 77% hybrid, and 6% to 86% *L. o. occidentalis*. The colony with the highest proportion of putative hybrids (77%) is East Sand Island (ESI17), located at the mouth of the Columbia River. This colony also contains equal proportions (11.5%) of *L. glaucescens* and *L. o. occidentalis*. The degree of putative introgression varies in the hybrid zone: north of Destruction Island (DES13), Washington, hybrids tend to be more *glaucescens*-like, south of East Sand Island (ESI17) at the mouth of the Columbia River, they tend to be more *occidentalis*-like (Bell 1992).

GENETIC RESULTS

GENETIC VARIABILITY

Of 32 loci scored across 33 colony areas (pooled taxa) within the *Larus glaucescens-occidentalis* complex, 25 (78%) were polymorphic. Of the polymorphic loci, 14 had two alleles each, 10 had three alleles, and one locus had four alleles. Seven loci were monomorphic (AAT-2, GDA, GPI, GTDH, MDH-2, SOD-1, and SOD-2).

The mean number of alleles per locus-colony

TABLE 4. Genetic variability measures of pooled taxa in 33 colony areas of the *L. glaucescens-occidentalis* complex. Percentage of polymorphic loci listed as ≤0.99 (P_{99}). Heterozygosities presented as direct count (H_{obs}).

Colony area	*n*	Mean no. alleles/ locus	P_{99}	H_{obs} ± (SE)
BULD1	10	1.3	28.1	0.044 (0.016)
KACH2	8	1.2	18.8	0.043 (0.018)
MIDD3	20	1.4	37.5	0.053 (0.016)
MASS4	9	1.3	21.9	0.049 (0.019)
CUMS5	33	1.4	40.6	0.045 (0.017)
STJA6	15	1.3	25.0	0.040 (0.014)
NVAN7	14	1.3	25.0	0.047 (0.020)
GEOR8	19	1.2	21.9	0.051 (0.021)
CLEL9	27	1.3	31.4	0.047 (0.018)
SJI10	29	1.4	37.5	0.051 (0.019)
TAT11	40	1.4	43.7	0.048 (0.015)
PRO12	17	1.2	18.7	0.035 (0.016)
DES13	18	1.3	28.1	0.052 (0.021)
GRA14	28	1.2	21.9	0.046 (0.018)
PUG15	15	1.3	25.0	0.054 (0.021)
WIL16	16	1.2	21.9	0.047 (0.019)
ESI17	27	1.4	43.7	0.049 (0.016)
TILL18	13	1.2	15.6	0.053 (0.023)
YAQ19	16	1.2	21.9	0.053 (0.023)
COO20	30	1.3	25.0	0.045 (0.018)
CCY21	16	1.1	12.5	0.039 (0.020)
TRN22	16	1.2	18.8	0.061 (0.027)
SUG23	18	1.2	18.8	0.057 (0.023)
MEN24	15	1.2	18.8	0.058 (0.027)
PTR25	13	1.2	18.8	0.053 (0.023)
FAR26	15	1.4	40.6	0.079 (0.026)
SFB27	21	1.2	21.9	0.062 (0.028)
ANO28	6	1.2	21.9	0.068 (0.025)
MOS29	11	1.2	18.8	0.057 (0.023)
MOR30	13	1.2	15.6	0.043 (0.020)
ANA31	32	1.3	25.0	0.063 (0.024)
SNC32	19	1.2	21.9	0.062 (0.026)
LCO33	10	1.2	15.6	0.078 (0.039)
Mean	18.4	1.3	24.9	0.052

area ranged from 1.4 to 1.1 and tended to be lower in colony areas south of the hybrid zone, i.e., south of COO20 (Table 4). Percent polymorphism (*P*) ranged from 12.5% at a *L. o. occidentalis* colony in northern California (CCY21) to 43.7% at two colonies within the hybrid zone (TAT11 in Washington and ESI17 in Oregon). Observed heterozygosity (*H*) ranged from 0.035 at PRO12 in Washington to 0.079 at FAR26 in California.

GEOGRAPHIC VARIATION

The EST-2 locus illustrates a typical pattern of allelic variation in the *Larus glaucescens-occidentalis* complex (Fig. 5). In EST-2, the "a" allele is found in all populations, but tends to be fixed

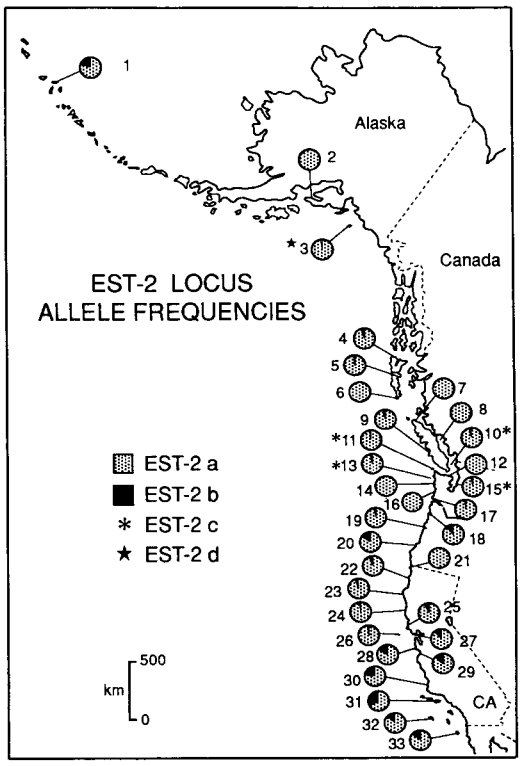

FIGURE 5. Pie diagrams illustrating allelic frequency variation at the EST-2 locus across all 33 colony areas, morphotypes pooled. Frequencies of the rare alleles EST-2^c and EST-2^d are less than 0.033.

in northern colonies and decreases in frequency southwards. The EST-2^b allele varies in a mosaic-like fashion across all colonies, but tends to increase in frequency southwards. The EST-2^c allele is a rare allele found in four colonies in the hybrid and introgressive zones (SJI10, TAT11, DES13, and PUG15). A rare allele is defined here as an allele that is found in one or more, but not all, colonies (see also Slatkin 1985). Another rare allele, EST-2^d, appears only at MIDD3 (*L. glaucescens*). Allelic frequency differences between colonies were not great enough to demarcate exact geographic limits for the hybrid and introgressive zones.

Principal components analysis of allelic frequency variation across all 33 colonies with morphotypes pooled indicate that colonies containing *L. glaucescens* occupy more multivariate space than those containing *L. o. occidentalis*, implying that *L. glaucescens* is genically more variable than the other members of the complex (Fig. 6). Note

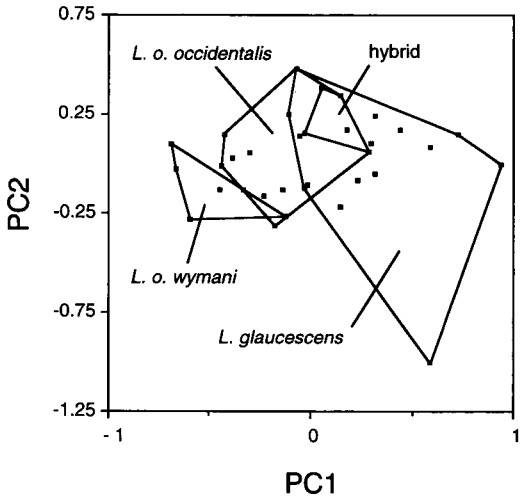

FIGURE 6. PCA of transformed allelic frequencies in seven variable loci across 33 colony areas with pooled morphotypes (see text). Labeled polygons enclose colony areas where each morphotype was collected; the polygon labeled hybrid includes only those colonies comprised of more than 50% hybrids. PC 1 and 2 account for 73% of the variation in transformed allelic frequencies.

that colonies containing more than 50% hybrids fall within the multivarite space of both putative parentals.

The presence of rare alleles may indicate hybridization-induced variability (Woodruff 1989). Grouping colony areas into regions based on their relationship to the hybrid zone revealed that the average number of rare alleles per colony differed significantly between regions ($F = 3.97$, $P = 0.029$, df = 2, 30). Colonies in Alaska, British Columbia, and Washington (BULD1 to SJI10, plus PUG15) averaged 3.82 rare alleles per colony, colonies in the hybrid zone in western Washington and northern Oregon (TAT11 to COO20, excluding PUG15) averaged 2.44, and south of the hybrid zone, in pure *L. occidentalis* colonies (CCY21 to LCO33), the average was 1.15 rare alleles per colony. A general trend of increasing allelic diversity from one region of parentals (*L. occidentalis*) through the hybrid zone into the other region of parentals (*L. glaucescens*) suggests that introgression is unidirectional, in this case, directed towards *L. glaucescens*.

GENETIC POPULATION STRUCTURE AND GENE FLOW

Rogers' (1972) and Nei's (1978) genetic distance measures revealed low overall intercolony distances (data in Bell 1992; tables of intercolony genetic distances are available from the author). Roger's D ranged from 0.007 (e.g., between TIL18 and MEN24) to 0.076 (between BULD1 and SNC32), and averaged 0.029 across all colony areas. Nei's D showed similar patterns, but lower overall values. The mean intercolony genetic distance was 0.003, and ranged from 0.000 (between KACH2 and MIDD3) to 0.028 (between BULD1 and SNC32).

Across all 33 colony areas with taxa pooled, single locus estimates of F_{ST} were low, but significant, for 18 of 25 polymorphic loci, and Wright's F_{IS}, a measure of potential inbreeding within populations (Wright 1951, 1965), was significantly greater than zero at seven loci (Table 5). Thus, although mean F_{ST} for all loci implies that only 8.2% of the total genetic variance is distributed among populations (Table 6), the *L. glaucescens-occidentalis* complex does not ap-

TABLE 5. Summary of F-statistics at the 25 variable loci found within the *L. glaucescens-occidentalis* complex across all 33 colony areas, morphotypes pooled. Significance levels for chi-square tests $H_0: F_{IS} = 0$, and $H_0: F_{ST} = 0$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Locus	F_{IS}[a]	F_{IT}	F_{ST}[b]
EST-1	−0.055	−0.004	0.048**
EST-2	0.091*	0.187	0.105***
EAP-1	−0.054	0.025	0.075***
EAP-2	−0.117**	−0.008	0.098***
PGDH	−0.032	−0.003	0.028
ACOH	−0.036	−0.004	0.031
LA	−0.017	0.028	0.045**
LGG	0.181***	0.214	0.040*
PAP	0.135***	0.219	0.098***
AAT-1	−0.019	−0.001	0.018***
ICD-1	0.030	0.179	0.154***
ICD-2	−0.035	−0.001	0.032***
MDHP	−0.018	0.076	0.093***
G3PDH	−0.023	0.048	0.070***
MDH-1	−0.017	0.000	0.017***
LDH	0.395***	0.435	0.067***
PGM-2	−0.013	0.120	0.132***
PGM-1	−0.022	0.051	0.071***
ADA	0.094*	0.171	0.085***
MPI	−0.024	−0.003	0.020
ACP	−0.026	0.000	0.025
ALD	−0.035	−0.001	0.032
ADH	0.095*	0.164	0.076***
CK-1	−0.016	−0.001	0.015
CK-2	−0.037	−0.007	0.029

[a] Chi-square = $F_{IS}^2 N(k-1)$, df = $[k(k-1)]/2$ (Waples 1987), where N is the total number of individuals sampled and k is the number of alleles at each locus.
[b] Chi-square = $2NF_{ST}(k-1)$, df = $(k-1)(s-1)$ (Waples 1987), where N and k are given above, and s is the total number of populations sampled.

TABLE 6. Mean values for F-statistical analyses of colony areas within the *L. glaucescens-occidentalis* complex, morphotypes pooled.

Colony areas	F_{IS}	F_{IT}	F_{ST}
All 33 colony areas (BULD1 to LCO33)	0.032	0.112	0.082
Introgressive and hybrid zones (CUMS5 to COO20; 16 colonies)	0.066	0.108	0.045
Hybrid zone (TAT11 to COO20, -PUG15; 9 colonies)	0.099	0.132	0.036

pear to be panmictic. Restricting the F-statistical analysis of colony areas with taxa pooled to most of the hybrid zone increases mean F_{IS} from 0.032 to 0.099 (Table 6), suggesting that inbreeding is higher in mixed-taxon colonies within the hybrid zone than in single-taxon colonies outside of the hybrid zone.

The estimated number of immigrants per colony generation (Nm_{est}) for pooled taxa is 2.80. This value is consistent with moderately high rates of gene flow and agrees with low, yet significant, levels of overall genic heterogeneity among colonies.

Chi-square goodness-of-fit and exact significance probability (Haldane 1954) tests detected departure from Hardy-Weinberg in several loci and colony areas (Table 7). Of these, the PAP locus was deficient in heterozygotes at three hybrid zone colonies (TAT11, GRA14, ESI17) and one *L. o. occidentalis* colony (CCY21). Although one or two departures may be expected due to chance (Cooper 1968), four significant departures from Hardy-Weinberg, three of which are in the hybrid zone, implies that mixed-taxon colonies are themselves not panmictic. Within the hybrid zone, the PAP[a] allele differs in frequency between *L. glaucescens* and *L. o. occidentalis* by 27% (Bell 1992), an amount which suggests that mating between the two taxa is not random, or at the very least, that gene flow between them is restricted.

TABLE 7. Presumptive genetic loci at colony areas that reveal significant departure from Hardy-Weinberg equilibrium as indicated by either the chi-square goodness-of-fit test or the exact significance probability (Pr) test (Haldane 1954), morphotypes pooled (Table 1). Four colony areas, TAT11, PRO12, GRA14, and ESI17, are located within the hybrid zone. Fixation Index (F) measures the standard deviation of the observed heterozygote frequency from the frequency expected under Hardy-Weinberg (Hedrick 1983). Here, positive values for F indicate heterozygote deficiency.

Colony area	Locus	Chi-square[a]	Pr[b]	F
BULD1	LDH	19.059**	0.053	1.000
TAT11	PAP	7.565	0.011*	0.419
	ADA	12.154**	0.075	0.474
PRO12	LA	5.880	0.038*	0.547
	MDHP	5.067	0.045*	0.514
GRA14	PAP	6.618	0.016*	0.467
ESI17	LGG	53.020**	0.019*	1.000
	PAP	4.743	0.050*	0.400
CCY21	PAP	9.006	0.007*	0.709
ANA31	EST-2	4.614	0.047*	0.362
LCO33	MDHP	9.000	0.007*	−1.000

[a] ** = $P < 0.0015$; modified significance level in simultaneous chi-square tests (Cooper 1968), see text.
[b] * = $P < 0.05$.

INTERSPECIFIC GENETIC DIFFERENTIATION

There were no fixed allelic differences between *a posteriori*-classified taxa of the *L. glaucescens-occidentalis* complex, that is, they all shared at least one allele per locus (data in Bell 1992, tables of allelic frequency data are available from the author). Although heterozygosity is highest in *L. o. wymani*, *L. glaucescens* appears to be the most variable gull in the complex because it has the highest average number of alleles per locus and the greatest percent polymorphism (Table 8).

Rogers' (1972) genetic distance between morphotypes of the *L. glaucescens-occidentalis* complex averaged 0.022, and ranged from 0.011 (between *L. glaucescens* and hybrids) to 0.037 (between *L. o. wymani* and *L. glaucescens*). Nei's D (1978) showed the same trend, albeit at much

TABLE 8. Genetic variability measures in members of *L. glaucescens-occidentalis* complex. Percentage of polymorphic loci listed as ≤0.99 (P_{99}). Heterozygosity is based on direct count (H_{obs}).

Taxon	*n*	Mean no. alleles/ locus	P_{99}	H_{obs} ± (SE)
L. glaucescens	217	1.9	43.7	0.046 (0.015)
Hybrids	128	1.7	37.5	0.049 (0.017)
L. o. occidentalis	184	1.6	25.0	0.056 (0.022)
L. o. wymani	84	1.3	25.0	0.061 (0.024)

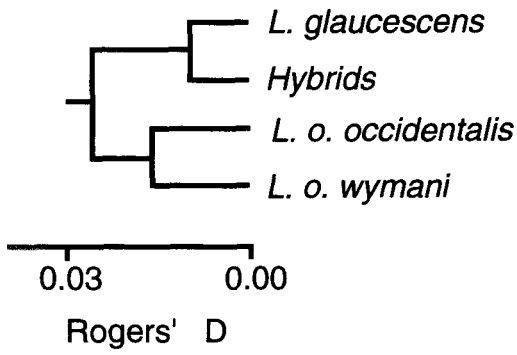

FIGURE 7. UPGMA cluster analysis of the *L. glaucescens-occidentalis* complex, based on a matrix of Rogers' (1972) genetic distances (r_{cc} = 0.90, %SD = 21.75). Taxa (= morphotypes), excluding *L. o. wymani*, were classified *a posteriori* by canonical DFA using colorimetric characters. Tree rooted at the midpoint. Sample sizes are given in Table 8.

lower values (Bell 1992). Hybrids cluster with *L. glaucescens* in a UPGMA phenogram of Rogers' D, indicating that they are genetically more similar to *L. glaucescens* than to *L. occidentalis* (Fig. 7).

DISCUSSION

PHENOTYPIC VERIFICATION OF HYBRIDS

Demonstration of phenotypic intermediacy is strong evidence in support of hybridization (Grant and Grant 1994). Although extensive overlap in allozymic and morphometric characters between *L. glaucescens* and *L. occidentalis* precluded their use in unequivocally identifying putative hybrids, discriminant function analysis, based on colorimetric traits, separated hybrid morphotypes as a group intermediate in canonical space between parental morphotypes. These results support the hypothesis that birds intermediate in coloration are in fact hybrids between *L. glaucescens* and *L. occidentalis*.

In many species of gulls, hybrids have been identified largely on the basis of plumage melanism and its presumed intermediacy in intergrades (Patten and Weisbrod 1974, Strang 1977, Ingolfsson 1970, 1987, Hoffmann et al. 1978, Firsova and Levada 1982, Spear 1987). As an alternative hypothesis, Snell (1991b) has pointed out that phenotypic intermediacy can be explained by intraspecific clinal variation. However, known crosses between gull species confirm that mantle grayness in F_1 hybrids is intermediate between the parentals (Harris et al. 1978) and that inheritance of primary tip melanism is probably continuous and dominant to nonpigmented tips (Lönnberg 1919). Mantle and primary tip melanism appear to be under separate genetic control: Galusha (pers. comm.) photographed a partially leucistic *L. glaucescens* that had an all white mantle, yet normally pigmented primary-tips. In the *Larus glaucescens-occidentalis* complex, mantle and primary tip melanism are among the best discriminators of pure and intergrade gulls, although the inclusion of several additional characters such as bill, eye-ring and iris pigmentation improve the accuracy of hybrid determinations.

RELATIVE VARIABILITY

The low level of genetic variability detected in the *L. glaucescens-occidentalis* complex is comparable to that found in other larids (Tegelström et al. 1980, Johnson 1985, Karl et al. 1987, Randi and Spinna 1987, Snell 1991a). However, within the complex, *L. glaucescens* has a higher percentage of polymorphic loci and its colonies occupy more space in a multivariate plot of gene frequencies than other members of the complex. In addition, *L. glaucescens* colonies located north of the hybrid zone have significantly more rare alleles than colonies to the south. Thus, *L. glaucescens* appears to exhibit greater allelic diversity than the other members of the complex, including hybrids. This seems to contradict the theory of contact zones wherein hybrids are expected to reveal greater electromorphic variability than parentals due to an increase in heterozygosity, an increase in the appearance of rare alleles (= hybrizymes), and an increase in the mutation rate (Barton et al. 1983, McClintock 1984, Woodruff 1989). Since *L. glaucescens* may hybridize with up to four other species of gull, its elevated electromorphic variability may simply be a reflection of extensive introgression originating from the four geographically disparate hybrid zones, and this introgression may be expressed as diffusing alleles and hybrizymes arising *de novo* through intragenic recombination and mutation. The amount of genetic variation observed in any one hybrid zone is less than the overall variability of the introgressed parental species.

CLINAL VARIATION AND GENE FLOW

In the *L. glaucescens-occidentalis* complex, size-related characters exhibit little, if any, clinal variation. *Larus glaucescens* from the Aleutian Islands is significantly larger than conspecifics from elsewhere, and this may be due in part to introgressive hybridization with a larger species, *L. hyperboreus,* in the Bering Sea region (Strang 1977). Specimens of *L. o. wymani* from the Channel Islands tend to be the smallest gulls in the complex (Bell 1992). Plumage melanism and eye-ring color vary in a stepwise manner across geography. Abrupt changes in color character means over relatively short geographic distances are found both within and between morphotypes, implying that gene flow may be restricted between the groups in question (Endler 1977). Although it is very difficult to demonstrate whether a given cline has been shaped by the process of natural selection or genetic drift (Endler 1982), concordant steps in several colorimetric characters along the western Washington coast suggests that this region represents a secondary contact zone between *L. glaucescens* and *L. occidentalis.*

Variable loci in the *L. glaucescens-occidentalis* complex exhibit shallow clinal variation across broad geographic distances. Shallow clines across great distances imply that alleles are diffusing freely and are not subject to selection of any great magnitude or, if selection is operative, that random genetic drift is obscuring its effects (Barton and Hewitt 1989). Because of the broad nature of the allelic clines and erratic shifts in allelic frequencies between islands, the exact location of the hybrid and introgressive zones in the *L. glaucescens-occidentalis* complex could not be pinpointed with protein electrophoretic methods.

Estimates of gene flow between populations of the *L. glaucescens-occidentalis* complex are comparable to those of other gulls (see Snell 1991a). The estimate obtained here for *Nm* (2.99 immigrants per generation) indicates moderately high gene flow. Nevertheless, under the stepping-stone model, which best approximates the linear arrangement of colonies in the *L. glaucescens-occidentalis,* gene flow rates of between 1 and 4 per deme still permit significant differentiation to occur (Kimura and Maruyama 1974).

As Snell (1991a) points out, a pattern of moderately high gene flow is consistent with other non-exclusive hypotheses: (a) limited gene flow coupled with minimal genetic divergence and extensive sharing of ancestral polymorphisms; (b) an average rate of genomic evolution and recent divergence; (c) a low rate of genomic evolution and ancient divergence. Prager and Wilson (1975) suggest that birds have lower rates of chromosomal and regulatory evolution than mammals. The lower rate of protein divergence in birds is thought by some to be a consequence of physiological constraint predicated by the higher avian body temperature (Avise and Aquadro 1982). Minimal protein divergence in birds may also be due in part to recent common ancestry. Snell (1991a, 1991b) hypothesizes a recent origin for several North Atlantic larids, a fact seemingly confirmed by Wink et al. (1994), who used mitochondrial DNA sequence analysis to estimate divergence times of 100,000 to 500,000 years ago for taxa in the *Larus cachinnans-fuscus-argentatus* superspecies group. Panov (1989) has argued that genetic divergence in birds is low because hybridization is a frequent response to changing environmental conditions (see also Pierotti and Annett 1993).

Genetic variability and cluster analyses suggest that electromorphic introgression is skewed towards *L. glaucescens.* Colorimetric data support the notion that introgression across the hybrid zone is largely unidirectional: whereas the southern borders of the hybrid and introgressive zones coincide, the northern borders do not. The zone of introgression extends much further north into the range of *L. glaucescens.* The evidence implies that *L. glaucescens* is becoming more introgressed than *L. o. occidentalis.* However, because several loci within the hybrid zone depart from Hardy-Weinberg proportions and F statistical analyses point to greater inbreeding within the hybrid zone, gene flow across the hybrid zone is clearly impeded. Departure from Hardy-Weinberg is sometimes considered to be a consequence of the Wahlund Effect, that is, a lack of heterozygotes due to the recent mixing of isolated populations of differing allelic frequencies (Hedrick 1983). In this case, Wahlund's Effect is not operative because the populations in question have been hybridizing to varying degrees at least since the early 1900's (Dawson 1909). Thus, the *L. glaucescens-occidentalis* hybrid zone corresponds to a partial barrier to gene flow, and the gene flow that does occur is largely skewed towards *L. glaucescens.* Skewed introgression is

manifested as greater variability in *L. glaucescens*. Patten (1980) hypothesized that gene flow may be proceeding from *L. argentatus* to *L. glaucescens* in southeastern Alaska, due in part to decreased fitness of hybrids at inland nesting areas favored by *L. argentatus*. I hypothesize that a similar mechanism may be operational in the *L. glaucescens-occidentalis* hybrid zone. Hybrids may experience reduced fitness in *L. o. occidentalis* habitat south of the hybrid zone. On a proximal level, hybrids could prefer *glaucescens*-habitat and consequently have a greater tendency to disperse into the fjord-like areas north of the hybrid zone.

In the genus *Manacus* secondary sexual traits have been found to spread unidirectionally across the hybrid zone between White-collared and Golden-collared Manakins (Parsons et al. 1993). Parsons et al. (1993) found that sexual selection may be responsible for driving male plumage traits across this hybrid zone despite impeded gene flow. The authors suggest that the hybrid zone may serve as a conduit for advantageous traits to spread between species without affecting the putative genetic integrity of the locally adapted populations or species. Although the selective basis is entirely unknown, an analogous situation may be occurring in the *L. glaucescens-occidentalis* complex. In spite of the fact that *L. glaucescens* is at population-genetic equilibrium (Slatkin 1993), it appears to exhibit elevated levels of hybridization-induced genetic variability.

STABILITY OF THE HYBRID ZONE

There are several caveats involved in directly comparing the surveys of Hoffman et al. (1978) with the present study. First, Hoffman et al. (1978) restricted their surveys of the hybrid zone to the outer Washington coast and Oregon; comparative data from British Columbia, the inland waterways of Washington and the south-central coast of Oregon are entirely lacking. Second, they used a hybrid index with varying levels of precision to categorize gulls in the field. Third, the only specimens they observed in the hand were trapped on Destruction Island and then released. Given these caveats, it nevertheless appears that the hybrid zone has expanded in the 10–15 year interval since the work of Hoffman et al. (1978). The proportion of intergrades at colonies in the hybrid zone has increased, and both parental species have expanded their breeding ranges across the zone. In the present study, *L. o. occidentalis* was collected as a breeding bird from colonies as far north as Tatoosh Island (TAT11) and as far east as Protection Island (PRO12) in Juan de Fuca Strait. *Larus glaucescens* was collected from nests as far south as Coos Bay, Oregon (COO20).

Although the hybrid zone appears to have expanded, it does exhibit elements of stability. The main region of sympatric overlap is still located along a 180 km portion of the outer Washington coast, the midpoint of the zone, located near Grays Harbor, Washington, has not shifted, and the relative proportion of morphotypes in colonies at the midpoint has remained the same. The fact that pure parentals of both species can be found at all colonies in the hybrid zone suggests that a hybrid swarm is not developing.

The present day overlap of *L. glaucescens* and *L. o. occidentalis* (up to 745 km of coastline) is much greater than it was in the early part of this century. Dawson (1908, 1909) noted mixed colonies of these two species along the outer Washington coast from Destruction Island north to Carroll Island, a distance of about 50 km. At that time the level of hybridization between these two species appears to have been minimal (Finley 1905, Dawson 1908, 1909). Furthermore, the hybrid zone does not seem to have changed much in the first half of this century (see Jewett et al. 1953). It appears that the hybrid zone began to expand as gull populations increased after World War II, thanks to reduced persecution and an increase in food resources such as garbage and fisheries by-products (Reid 1988). In the last 50 years the population of *L. glaucescens* in British Columbia has increased 3.5-fold (Campbell et al. 1990), while *L. o. occidentalis* has experienced population increases along the outer Washington coast (Speich and Wahl 1989). Thus, expansion of the hybrid zone in the latter half of this century may be a direct consequence of increasing gull populations.

ZOOGEOGRAPHY AND INFERRED PLEISTOCENE SCENARIOS

The present-day breeding distribution of *L. glaucescens* maps a marine environment that is characterized by complex fjord and estuarine circulation patterns and is subject to fluctuating salinity gradients (Thomson 1989). The hybrid zone between *L. glaucescens* and *L. occidentalis* occurs at the transition between two distinct marine-zoogeographical provinces—the northern Aleutian and southern Oregon provinces (Uda-

vardy 1978). In contrast to the Aleutian Province, the cold-temperate Oregonian Province is influenced by the southward-progressing California Current (Seapy and Littler 1980). The California Current, together with the wind-induced upwelling so characteristic for the Oregon and California coasts in spring and early summer, act to greatly increase ocean productivity (Huyer 1983). The distribution of *L. o. occidentalis* maps exactly the coastal upwelling ecosystem of western North America. The close correspondence between population distribution and marine-zoogeographical province implies that each of these gull taxa have adapted to and tracked different environmental conditions during their evolution.

Most of the area where present-day *L. glaucescens* and *L. occidentalis* overlap was inundated by the southern portions of the Cordilleran Ice-sheet at the height of the Late Wisconsin Drift in North America, roughly 18,000 yr B.P. (Waitt and Thorson 1983). The ice sheet covered all of Vancouver Island and Juan de Fuca Strait extending beyond Cape Flattery and filling the entire Puget Sound basin. Since very few larids are adapted to a sea ice environment (Divoky 1979), the distributions of these two species were probably separated by glacial fronts. *Larus glaucescens* was likely situated to the north, in ice-free coastal refugia postulated for the Bering Sea coast, Kodiak Island or the Queen Charlotte Islands (Lindroth 1969, Heusser 1989); while *L. occidentalis* may have been forced well south of Juan de Fuca Strait (see also Hoffman et al. 1978). Global sea level changes ranging up to 100m during glacial maxima (Clague 1989) may have created additional refugia on the continental shelf or coastal seamounts. As glacial meltdown progressed from 18,000 to 12,000 yr B.P., the Columbia River was subject to catastrophic flooding (Bretz 1923). Flooding was so great that it likely wiped out gull nesting habitat in western Washington. It may also have temporarily eliminated coastal upwelling. In effect, the Columbia River could have acted as a barrier to northward range expansion by *L. occidentalis* at the beginning of the Holocene. Contact between the two species was probably not established until well after 10,000 yr B.P., when ocean waters began filling Puget Sound (Waitt and Thorson 1983).

The geological history of the Pacific Northwest implies then, that *L. glaucescens* and *L. occidentalis* may have gone through multiple bouts of secondary contact followed by separation. Thus, hybridization probably occurred during each contact episode, but did not proceed far enough to swamp either species. Habitat alteration by humans in the last century may have further promoted contact between these two species, and each bout of hybridization may have effectively increased the genetic diversity in at least one of the hybridizing taxa—*L. glaucescens*. The *L. glaucescens-occidentalis* complex may thus serve as a prime example of the role hybridization plays in enabling gene exchange to occur between species and thereby increase genetic variability.

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APPENDIX 1

Loci examined and electrophoretic conditions employed in this study. Loci are listed by name (Hillis and Moritz 1990) and abbreviation, followed by E. C. Number, and electrophoretic conditions, where [1] = Amine citrate buffer: gel pH 6.0, electrode pH 6.1, run @ 75 mA, 5 h. [2] = Tris-citrate buffer: gel pH 8.0, electrode pH 8.1, run @ 130 V, 4 h. [3] = Lithium hydroxide buffer: gel pH 8.2, electrode pH 8. 1, @ 300 V, 3 h.

Aconitase Hydratase (ACOH), EC 4.2.1.3, [1]; Acid Phosphatase (ACP), EC 3.1.3.2, [1]; Adenosine Deaminase (ADA), EC 3.5.4.4, [2]; Alcohol Dehydrogenase (ADH), EC 1.1.1.1, [1]; Aldolase (ALD), EC 4.1.2.13, [1]; Aspartate Aminotransferase (AAT 1,2), EC 2.6.1.1, [1]; Creatin Kinase (CK 1, 2), EC 2.7.3.2 [1]; Erythrocytic Acid Phosphatase (EAP 1, 2), EC 3.1.3.2 [1]; Esterase (EST 1, 2) EC 3.1.1.-, [1]; Guanine Deaminase (GDA), EC 3.5.4.3, [3]; Glutamate Dehydrogenase (GTDH), EC 1.4.1.2, [2]; Glycerol-3-phosphate Dehydrogenase (G3PDH), EC 1.1.1.8, [2]; Glucose-6-phosphate Isomerase (GPI), EC 5.3.1.9 [1]; Isocitrate Dehydrogenase (ICD 1, 2), EC 1.1.1.42, [1]; Leucyl-alanine Dipeptidase (LA), EC 3.4.-.-, [3]; Lactate Dehydrogenase (LDH), 1.1.1.27, [1]; Leucyl-glycylglycine Tripeptidase (LGG), EC 3.4.-.-, [3]; Malate Dehydrogenase (MDH 1, 2), EC 1.1.1.37, [1]; Malate Dehydrogenase NADP+ (MDHP), EC 1.1.1.40, [1]; Mannose-6-phosphate Isomerase (MPI), EC 5.3.1.8, [2]; Purine-nucleoside Phosphorylase (PNP), EC 2.4.2.1, [2]; Phenylalanyl-proline Dipeptidase (PAP), EC 3.4.-.-, [3]; Phosphoglucomutase (PGM 1, 2), EC 5.4.2.2, [1, 2]; Phosphogluconate Dehydrogenase (PGDH), EC 1.1.1.44, [1]; Superoxide Dismutase (SOD 1, 2), EC 1.15.1.1, [1].