INTERSPECIFIC ALLOZYME DIFFERENTIATION AMONG NORTH ATLANTIC WHITE-HEADED LARID GULLS

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ABSTRACT.—I assessed patterns of allozymic variation in *Larus argentatus* (Herring Gulls), *L. cachinnans* (Yellow-legged Herring Gulls), *L. fuscus* (Lesser Black-backed Gulls), *L. glaucoides* (Iceland Gull), *L. hyperboreus* (Glaucous Gulls), and *L. marinus* (Greater Black-backed Gull). I surveyed 34 presumed structural gene loci over 479 individuals. All birds were monomorphic and fixed for the same allele at each of 26 loci. There were neither fixed allelic differences nor marker alleles for the different species across eight polymorphic loci. Quantitative gene-flow estimates are suggestive of high interspecific gene flow (Nm = 2.1–10.7 per generation). Gene-diversity analyses provide evidence that almost all allozyme variation (>90%) occurs within colonies, whereas almost none (2.3%) is accounted for by diversity among species.

All these species are similar in both protein encoding genes and morphology, which implies overall genetic similarity. My results are consistent with the hypothesis that these taxa are of very recent origin, and indicate that speciation need only involve a minute portion of the total genome. Additional genetic differentiation such as the accumulation of rare alleles or fixed allelic differences at electrophoretically detectable loci may simply be an artifact of the time elapsed subsequent to speciation. *Received 27 March 1990, accepted 16 October 1990.*

STUDIES of hybridization are important in attempts to understand the processes of population differentiation and, ultimately, speciation (Barton and Hewitt 1985). Among colonially breeding seabirds, hybridization is thought to be common (Pierotti 1987 and references therein), especially among gulls in the genus *Larus*. Pierotti (1987) argued that the specific mate-recognition systems (*sensu* Paterson 1980, 1981) of seabirds are exceedingly simple and are cued to critical morphological characters of leg/foot coloration, bill pattern, and perhaps size. Where recognition systems of different populations overlap, range expansion and secondary contact may be followed quickly by hybridization. In general, hybridization is expected wherever gulls of different taxa with similar leg/foot coloration and bill patterns are sympatric.

Analysis of apparent hybrid zones in gulls is complicated by the incomplete descriptions of natural variation of allopatric populations. The earlier conclusions (Smith 1966a, 1969) on the extent of hybridization in some charadriiform birds appear unreliable. Unfortunately, completing the published set of experimental protocols on which Smith's (1966a, b; 1969) conclusions were reportedly based would have been logistically impossible (Snell 1989). Regardless, in east Iceland, secondary contact between *Larus argentatus* (Herring Gull) and *L. hyperboreus* (Glaucous Gull) occurred during the 1920s following range expansion of *argentatus*. Variable and seemingly intermediate plumage patterns among Icelandic *argentatus* are suggestive of *argentatus* × *hyperboreus* hybridization (Ingolfsson 1970, 1987), though morphometric evidence (Snell 1991) indicates that these patterns simply reflect intraspecific variability.

I used protein electrophoresis to evaluate genetic differentiation in *L. argentatus*, *L. cachinnans* (Yellow-legged Herring Gulls), *L. fuscus* (Lesser Black-backed Gulls), *L. glaucoides* (Iceland Gull), *L. hyperboreus*, and *L. marinus* (Great Black-backed Gull). (*L. cachinnans* are considered *argentatus michahellis* by some authors.) I evaluate evidence for (1) patterns of genetic structuring among populations, (2) *argentatus* × *hyperboreus* hybridization in Iceland, (3) genetic differentiation by distance, and (4) gene flow.

METHODS

I collected tissue samples of *argentatus*, *cachinnans*, *fuscus*, *glaucoides*, and *hyperboreus* from 14 populations in Europe and North America (Table 1, Fig. 1). I analyzed an additional composite sample of *L. marinus* from eastern Iceland, eastern Canada, and northern France (n = 5). Tissue samples of heart, liver, and pectoral muscle for each bird (total n = 479 gulls)

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TABLE 1. *Larus* gull sample sites. Sample sizes (males and females pooled) indicated.

Locality	Latitude	Longitude	Species	*n*	Year
1 Home Bay, Baffin Island, Canada	69°00'N	68°00'W	*hyperboreus*	47	1985
2 Svalbard (Longyearbyen)	78°14'N	15°39'E	*hyperboreus*	48	1987
3 Bjarnjarhafnjarfjall, western Iceland	65°00'N	23°00'W	*hyperboreus*	53	1986
4 Skruder, east Iceland	64°54'N	13°37'W	*argentatus*	61	1986
5 Karlsskali, east Iceland	65°01'N	13°40'W	*argentatus*	15	1986
6 Kent Island, New Brunswick, Canada	44°35'N	65°22'W	*argentatus*	32	1986
7 P. E. I. (Malpeque Bay), Canada	46°30'N	63°50'W	*argentatus*	31	1986
8 Tromsö, Norway	69°42'N	19°00'E	*argentatus*	51	1987
9 Île de Balanec, Bretagne, France	48°25'N	4°59'W	*argentatus*	23	1988
10 Île Ricard, Bretagne, France	48°41'N	3°54'W	*argentatus*	30	1987
11 Camargue (Étang du Fournelet), France	43°21'N	4°40'E	*cachinnans*	34	1988
12 Camargue (Étang de la Galère), France	43°29'N	4°37'E	*cachinnans*	16	1988
13 Faroe Islands (Torshavn)	62°02'N	6°47'W	*fuscus*	24	1987
14 Home Bay, Baffin Island, Canada	69°00'N	68°00'W	*glaucoides*	9	1985
15 Composite from sites 4, 6, and 9; see text			*marinus*	5	—

were quick-frozen in liquid nitrogen in the field (no pectoral muscle was retained from Home Bay birds). Tissues were later kept either in liquid nitrogen or in a −80°C freezer.

As genetic variability and differentiation among gulls was known to be slight (Ryttman et al. 1980; Tegelstrom et al. 1980; Johnson 1982, 1985; Zink and Winkler 1983), I sampled populations from geographically distant regions to increase the likelihood of detecting genetic differentiation. Birds were collected during the breeding season by shooting, trapping, or drugging with either tribromoethanol or alpha-chloralose. Where possible, I collected birds at breeding colonies. Otherwise, gulls were obtained at refuse dumps near active breeding sites.

The nuclear genome of *Larus* populations was examined by horizontal starch-gel electrophoresis. A total of 34 presumptive loci were resolved (Appendix). Techniques followed Shaw and Prasad (1970) and Harris and Hopkinson (1976). Tissues were prepared for each bird by joint homogenization in a 0.1 M Tris-HCl buffer (pH 7.0) for most enzymes. I used a 0.01 M dithiothreitol-0.1 M Tris-HCl buffer (pH 6.0) for peptidases. Tissue homogenates were centrifuged for 2 min in a Beckman microcentrifuge, and supernatants stored at −80°C until used. Running conditions and gel/buffer combinations were standard and are available on request. I used 9–10% starch in all gels. Histochemical stain recipes generally followed those in Shaw and Prasad (1970), Clayton and Tretiak (1972), Turner (1973), Harris and Hopkinson (1976) or Smith (1976); see St. Louis (1986: appendix 3) for additional stain recipes. Isozymes, when present, were numbered sequentially from cathode to anode. Allozymes were scored by letters beginning with *A*, in decreasing order of frequency.

Of the 34 loci scored, eight varied within or among gull populations (see Results). All individuals were scored for all loci to obtain frequency estimates of rare alleles, which provide information on gene flow (Slatkin 1985, Barton and Slatkin 1986). Sequential electrophoresis of all individuals (Aquadro and Avise 1982, Hackett 1989) was not performed. However, initial analyses evaluated resolution of the different enzyme systems under various buffer and gel conditions. Gels for any particular enzyme system run under differing electrophoretic conditions did not differ in either the number of alleles present or their relative patterns.

I used BIOSYS-1 (Swofford and Selander 1989) to calculate allele frequencies, measures of genetic variability (mean heterozygosity by direct count and Hardy-Weinberg expectation), the exact significance of deviation from Hardy-Weinberg equilibrium within populations of individual loci (analogous to Fisher's exact test), *F*-statistics, and matrices of Nei's (1978) unbiased and Rogers' (1972) *D* genetic distance. I used NTSYS (Rohlf 1989) for permutation tests.

Gene-diversity analysis (Nei 1977: 231) partitioned total variance in the entire set of samples (H_T) as follows: $H_T = H_C + D_{CS} + D_{ST}$, where H_C and D_{CS} are the gene diversities within and between colonies within subdivisions, and D_{ST} is variation among subdivisions relative to the total. Gene-diversity values were based on hierarchical *F*-statistics (Swofford and Selander 1989: 21).

To estimate rates of gene flow among populations and species, I used Slatkin's (1981) indirect approach of relating mean $p(i)$ (mean frequency of alleles found in i of d demes) with i/d (number of populations in which allele is present/total number of populations). Also, *Nm* (the number of migrants between demes per generation) was directly estimated using the "private" allele approach of Slatkin (1985) and Barton and Slatkin (1986) and the F_{ST} method of Wright (1978).

Mantel's test (Dietz 1983) was used to evaluate the significance of the association between among-locality geographic great-circle distance (GCD°) and ge-

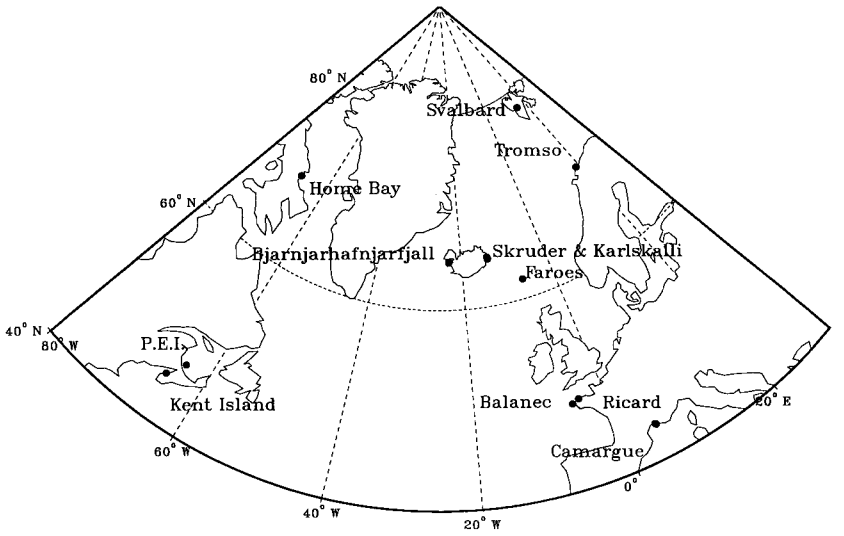

Fig. 1. Geographic map of sampling localities.

netic differentiation (Rogers' *D*). As Jackson and Somers (1989) provide evidence that Mantel's test is not stable with as few as 500 permutations, I used 10,000 to calculate significance levels. I evaluated genetic differentiation by distance interspecifically among the 14 populations of *argentatus, cachinnans, fuscus, glaucoides,* and *hyperboreus.* Geographic GCDs (in degrees of arc) were calculated as follows:

$$GCD° = \arccos[(\sin A \sin B) + (\cos A \cos B \cos P)],$$

where GCD° = great-circle distance between two geographic points, A and B; A = latitude of A; B = latitude of B; and P = difference in longitude between A and B. A program (written in QuickBasic 4.5) to calculate a lower triangular GCD° matrix from a series of latitude-longitude coordinates is available on request.

Results

General results of allozyme analyses.—Twenty-four enzyme systems representing 34 presumptive gene loci were resolved satisfactorily. Twenty-six loci were monomorphic and fixed for the same allele in all gulls (Appendix). Allele frequencies at the eight variable loci are shown in Table 2. Average number of alleles per locus per population varied from 1.0 to 1.3. Levels of polymorphism were low, ranging from 2.9% (*marinus*) to 20.6% (*argentatus* from Tromsö). Mean observed heterozygosity (H_{obs}) of individual populations was 0.044 or less. However, there was a consistent positive correlation (Spearman rank test) between sample size and the average number of alleles ($r = 0.46$, $n = 15$, NS), polymorphism level ($r = 0.53$, $n = 15$, $P < 0.05$), and, mean H_{obs} ($r = 0.38$, $n = 15$, NS). There is a tendency for larger samples to be more variable.

Standard errors of mean H_{obs} estimates were large, and in some instances they were as large as the estimates themselves. For each population the mean $H_{obs} \pm 1$ SE overlapped the mean Hardy-Weinberg expected value (H_{exp}). Mean H_{obs} were highest among *hyperboreus* populations from Svalbard (0.044 ± 0.018) and Home Bay (0.041 ± 0.021). Mean H_{obs} was reduced among conspecifics from Bjarnjarhafnjarfjall (0.013 ± 0.009). Among *argentatus* populations, mean heterozygosity was highest at Tromsö (0.023 ± 0.010); it was reduced at Malpeque Bay (0.018 ± 0.010), Île Ricard (0.017 ± 0.010), Île de Balanec (0.015 ± 0.007), and Kent Island (0.013 ± 0.010); and it was lowest at the Icelandic colonies of Skruder (0.012 ± 0.006) and Karlsskali (0.008 ± 0.005). Among *cachinnans,* mean heterozygosity was low at Étang du Fournelet (0.013 ± 0.008) and Étang de la Galère (0.009 ± 0.009). The mean heterozygosity estimates of *glaucoides* (0.029 ± 0.020), *fuscus* (0.009 ± 0.005), and the composite *marinus* sample (0.006 ± 0.006) were also low.

In only 6 of 60 locus-by-locus comparisons of individual populations did H_{obs} and H_{exp} differ significantly ($P \leq 0.05$, exact probabilities were calculated for each polymorphic locus in each

TABLE 2. Frequencies of alleles at eight polymorphic loci in 15 Larid populations. The sequence and sample size of populations are provided in Table 1. Loci are defined in the Appendix.

	Population						
	hyperboreus			*argentatus*			
Locus	1	2	3	4	5	6	7
EST-1 A	1.000	1.000	1.000	1.000	1.000	0.938	0.823
B	0.000	0.000	0.000	0.000	0.000	0.063	0.177
EST-2 A	0.830	0.875	0.943	1.000	1.000	1.000	1.000
B	0.170	0.125	0.057	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GDA A	0.915	0.833	0.991	0.926	0.933	1.000	0.952
B	0.085	0.167	0.009	0.074	0.067	0.000	0.048
GPD A	0.511	0.510	0.028	0.057	0.067	0.194	0.177
B	0.489	0.490	0.972	0.943	0.933	0.806	0.823
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH A	0.979	0.958	1.000	0.934	1.000	1.000	1.000
B	0.021	0.042	0.000	0.066	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGI A	0.787	0.865	0.811	0.984	0.933	0.969	0.968
B	0.213	0.135	0.189	0.016	0.067	0.031	0.032
PGD A	0.957	0.854	1.000	1.000	1.000	1.000	1.000
B	0.043	0.146	0.000	0.000	0.000	0.000	0.000
PGM A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000

population). In each case there was a deficiency of heterozygotes.

Genetic structuring among populations.—*F*-statistic analyses are typically confined to populations within a single species, even in multispecies comparisons (e.g. Waples 1987). I calculated *F*-statistics across all species to reflect the apparent frequency of interspecific hybridization among gulls (Pierotti 1987) and the presumed nonindependence of breeding pools.

I used standardized genetic variance (F_{ST}) to determine the position of both the entire set of populations and also populations among species relative to panmixia ($F_{ST} = 0$), and to complete fixation for alternative alleles ($F_{ST} = 1$). The F_{ST} estimate for each polymorphic locus was significant (Table 3). The mean F_{ST} (0.108), averaged across all 15 populations and all six species, indicated relatively little geographic and interspecific variation in allele frequency.

Mean F_{IS} (measure of inbreeding within populations) equaled 0.081, with all values for individual loci except GPD being positive (Table 3). Statistically significant F_{IS} values for EST-1 (0.408), EST-2 (0.198), and PGM (0.418) indicate a deficiency of heterozygotes. Such deficiencies might be caused by high levels of natal-site philopatry, or substructuring of populations even within colonies.

Gene-diversity.—Significant F_{ST} values implied geographic and interspecific variation in genomes. Gene-diversity analysis detailing patterns of allozyme differentiation (e.g. Grudzien et al. 1987) was done by separate hierarchical analyses of (1) the six species, (2) pink/gray and yellow-footed gulls, and (3) non-Icelandic *hyperboreus*, non-Icelandic *argentatus*, and Icelandic colonies containing apparent or possible *argentatus* × *hyperboreus* hybrids. Regardless of how the set of 15 populations was subdivided, the within-colony diversity (H_C) accounted for almost all allozymic variation (>90%) (Table 4).

Subdivision of populations by species provided evidence of diversity within taxa (D_{CS}) that accounted for 6.9% of the variation, whereas diversity among taxa (D_{ST}) accounted for only 2.3% of total genic variability. Subdivision of populations by leg color (probable mate recognition cues) provided evidence that diversity within mate recognition systems (D_{CS}) accounted for 9.9% of the variance. No genetic variance was assigned to diversity among mate-recognition systems, and in fact the D_{ST} estimate was slightly negative. Subdivision of populations

TABLE 2. Extended.

Population							
argentatus			*cachinnans*		*fuscus*	*glaucoides*	*marinus*
8	9	10	11	12	13	14	15
1.000	0.978	1.000	1.000	1.000	1.000	1.000	1.000
0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000
0.961	0.913	1.000	0.882	0.938	0.875	0.944	1.000
0.029	0.065	0.000	0.118	0.063	0.083	0.056	0.000
0.000	0.022	0.000	0.000	0.000	0.042	0.000	0.000
0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.931	0.978	0.783	1.000	1.000	1.000	1.000	1.000
0.069	0.022	0.217	0.000	0.000	0.000	0.000	0.000
0.196	0.130	0.150	0.191	0.156	0.021	0.333	0.000
0.804	0.870	0.817	0.809	0.844	0.979	0.667	1.000
0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000
0.912	0.978	0.950	1.000	1.000	0.979	1.000	1.000
0.078	0.022	0.050	0.000	0.000	0.021	0.000	0.000
0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.931	0.978	1.000	0.971	1.000	1.000	1.000	0.900
0.069	0.022	0.000	0.029	0.000	0.000	0.000	0.100
0.990	1.000	1.000	1.000	1.000	1.000	0.944	1.000
0.010	0.000	0.000	0.000	0.000	0.000	0.056	0.000
0.980	1.000	1.000	1.000	1.000	0.896	0.944	1.000
0.020	0.000	0.000	0.000	0.000	0.104	0.056	0.000

across the Icelandic *argentatus* × *hyperboreus* contact zone (*cachinnans, fuscus, glaucoides,* and *marinus* were excluded from this analysis) indicated gene diversity within allopatric, sympatric, and Icelandic subdivisions (D_{CS}) accounted for only 2.6% of the variance. Diversity among these subdivisions (D_{ST}) accounted for 7.1% of total genic variability across the contact zone.

Genetic and geographic distance.—Nei's *D* values are all extremely small (0.000–0.009), are comparable to values reported between other populations of gulls (e.g. Karl et al. 1987), and may not differ significantly from zero (Table 5). Nei's *D* values of zero occurred between populations within and among species, which indicates near genetic identity between these gull taxa. Similarly, Rogers' *D* distances were low (0.004–0.032). The Mantel's test provided no ev-

TABLE 3. Summary of *F*-statistics at all polymorphic loci. Significance levels are associated with Chi-square tests of (1) H_0: $F_{IS} = 0$, and (2) H_0: $F_{ST} = 0$; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Only populations polymorphic for the locus being tested were included in each analysis.

Locus	*F*-statistics		
	F_{IS}[a]	F_{IT}	F_{ST}[b]
EST-1	0.408***	0.480	0.122***
EST-2	0.198***	0.244	0.057***
GDA	0.052	0.132	0.085***
GPD	−0.032	0.131	0.157***
IDH	0.065	0.098	0.035***
PGI	0.113*	0.184	0.080***
PGD	0.068	0.150	0.088***
PGM	0.418***	0.458	0.069**
Mean	0.081	0.181	0.108

[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 from populations polymorphic for the locus being tested, and *k* is the number of alleles at that locus.

[b] Chi-square = $2NF_{ST}(k-1)$, df = $(k-1)(s-1)$ (Waples 1987), where *N* and *k* are defined above, and *s* is the number of populations polymorphic for the locus being tested.

TABLE 4. Gene-diversity estimates of six species of White-headed Gulls. Subdivisional groupings of populations analyzed separately.

Subdivisions	H_C	D_{CS}	D_{ST}
Species[a]	0.908	0.069	0.023
Leg color[b]	0.909	0.099	−0.008
Hybrid zone[c]	0.903	0.026	0.071

[a] *argentatus, cachinnans, fuscus, glaucoides, hyperboreus,* and *marinus.*

[b] Morphological cues in these birds' specific mate-recognition systems: gray/pink or yellow legs.

[c] Icelandic *argentatus* or *hyperboreus,* allopatric *argentatus,* and allopatric *hyperboreus.*

TABLE 5. Matrices of genetic distances among population samples of *Larus* gulls, with Nei's (1978) unbiased *D* above the diagonal and Rogers' (1972) *D* below. Populations are sequenced as in Table 1.

	hyperboreus			*argentatus*			
	1	2	3	4	5	6	7
1	—	0.000	0.007	0.008	0.007	0.005	0.006
2	0.010	—	0.008	0.008	0.007	0.005	0.006
3	0.022	0.028	—	0.001	0.000	0.002	0.002
4	0.027	0.028	0.011	—	0.000	0.001	0.001
5	0.025	0.027	0.008	0.004	—	0.000	0.001
6	0.026	0.028	0.013	0.010	0.009	—	0.000
7	0.028	0.031	0.017	0.012	0.010	0.005	—
8	0.021	0.023	0.014	0.008	0.008	0.009	0.012
9	0.023	0.026	0.010	0.008	0.008	0.007	0.010
10	0.027	0.024	0.019	0.008	0.011	0.012	0.013
11	0.021	0.023	0.012	0.012	0.010	0.005	0.011
12	0.024	0.027	0.010	0.009	0.008	0.006	0.010
13	0.030	0.032	0.011	0.011	0.012	0.015	0.019
14	0.020	0.022	0.018	0.018	0.017	0.012	0.017
15	0.028	0.030	0.005	0.008	0.005	0.010	0.014

idence of a significant association among the 14 populations of the five gull species between Rogers' *D* and great-circle distance ($Z = 0.14$, $P = 0.180$).

Gene flow.—I calculated an indirect measure of gene flow (i.e. the observed relation between mean $p[i]$ and i/d) from the allele frequency data from the six gull species (Table 2). The resulting curve was concave (Fig. 2), a pattern consistent with a hypothesis of high gene flow.

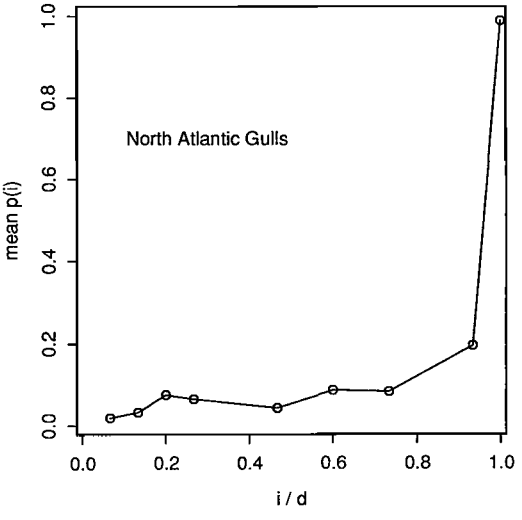

Fig. 2. Inverted-"L" relation of $p(i)$ to i/d, over 15 populations of *argentatus, cachinnans, fuscus, hyperboreus, glaucoides,* and *marinus*. This pattern is consistent with conflicting hypotheses of (1) high rates of gene flow and (2) little or no gene flow among minimally diverged populations sharing ancestral polymorphisms.

Based on Monte-Carlo simulations, Slatkin (1985) and Barton and Slatkin (1986) argued that the mean frequency of "private" alleles (i.e. alleles found in only one population) could provide an estimate of Nm, the actual number of migrants between demes per generation. Barton and Slatkin's (1986: 413) simulations suggest an almost linear relation between logarithm $p(1)$ and logarithm Nm, of the form $\log_{10}[p(1)] = [a \log_{10}(Nm)] + b$, where $0.01 < Nm < 10$. The mean sample size per population of these gulls was 31.9, and the mean frequency of "private" alleles [$p(1)$] in these gulls equaled 0.018. The coefficients a and b vary with mean sample size per population, and were interpolated between the values Barton and Slatkin provided (for $n = 25$, $a = -0.576$ and $b = -1.11$; for $n = 50$, $a = -0.612$ and $b = -1.21$) to $a = -0.586$ and $b = -1.14$. Thus, the estimate for Nm among the populations of the six species is 10.7 birds per generation.

An alternate estimate of Nm based on *F*-statistics (Wright 1978) was calculated as $Nm = ([F_{ST}^{-1}) - 1]/4)$. With the mean F_{ST} averaged across populations and loci (0.108, Table 3), the estimate for Nm was 2.1.

DISCUSSION

Genetic structuring.—If it reflected presumed genetic cohesiveness and monophyletic origin

TABLE 5. Extended.

argentatus			*cachinnans*		*fuscus*	*glaucoides*	*marinus*
8	9	10	11	12	13	14	15
0.004	0.005	0.006	0.004	0.005	0.009	0.002	0.009
0.004	0.006	0.005	0.004	0.005	0.009	0.002	0.009
0.001	0.001	0.003	0.001	0.001	0.001	0.003	0.000
0.001	0.000	0.001	0.001	0.000	0.001	0.002	0.000
0.000	0.000	0.001	0.001	0.000	0.001	0.002	0.000
0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001
0.001	0.001	0.002	0.001	0.001	0.002	0.001	0.002
—	0.000	0.001	0.000	0.000	0.001	0.000	0.001
0.009	—	0.001	0.000	0.000	0.000	0.001	0.000
0.011	0.012	—	0.002	0.001	0.002	0.002	0.002
0.009	0.005	0.013	—	0.000	0.001	0.000	0.001
0.009	0.004	0.011	0.004	—	0.001	0.000	0.001
0.016	0.009	0.018	0.011	0.009	—	0.002	0.001
0.014	0.013	0.018	0.010	0.009	0.015	—	0.003
0.013	0.010	0.016	0.011	0.009	0.010	0.018	—

of nominal gull species, intraspecific divergence among populations would be predicted to be less than interspecific divergence. I found no evidence of this. Interspecific genetic distances were in many instances less than intraspecific ones (e.g. Home Bay *hyperboreus* are more similar to sympatric *glaucoides* than to Bjarnjarhafnjarfjall conspecifics, and Ile de Balanec *argentatus* are more similar to *fuscus* from the Faroe Islands than to Ile Ricard conspecifics). White-headed gulls have differentiated so little that these electrophoretic data cannot be used to either evaluate alternative taxonomic hypotheses or reconstruct historical patterns of divergence. Similarly, these data provided no insight into genetic structuring among mate-recognition systems.

Genetic evidence for Icelandic argentatus × hyperboreus *hybridization.*—Hybrid populations are predicted to be more variable than allopatric ones both morphologically (Schueler and Rising 1976) and electrophoretically (Corbin 1981). There is no evidence Icelandic populations of either *argentatus* or *hyperboreus* are more variable than allopatric populations of either species morphologically (Snell 1991) or genetically (these data). However, Corbin's (1981) prediction assumes the parental taxa have themselves differentiated to a notable degree. As these species are not differentiated allozymically, the genetic data are not useful for testing Corbin's hypothesis.

Gene flow.—Reliable estimates of gene flow would be of considerable importance to understand the role of selection and environmental induction in the production and maintenance of phenotypic differentiation. Where actual levels of gene flow are high and populations are morphologically differentiated, nonselective and nongenetic factors are implicated directly as causal mechanisms in producing and maintaining phenotypic clines.

Slatkin (1981) argued the shape of the $p(i)$ vs. i/d relation could be used to estimate levels of gene flow among populations. Slatkin suggested that, whereas an "r"-shaped $p(i)$ vs. i/d pattern would provide evidence of low levels of gene flow (reflecting many "private" alleles occurring at very high frequency, including at or near fixation), a reversed-"L" shape would provide evidence of high rates of gene flow (absence of fixed allelic differences, coupled with few private alleles at very low frequency). On the basis of a reversed-"L" $p(i)$ vs. i/d pattern and evidence of morphological differentiation among populations, Zink (1986: 107) proposed "that local environmental conditions acting during the nestling period shape inherent phenotypic plasticity, effecting spatial patterns." Though this is possible, there is no reason to think morphological patterns in these gulls simply reflect proximate environmental conditions. Species are morphologically distinct even where they breed sympatrically (e.g. *argentatus, fuscus,* and *marinus* on and near Ile Balanec, or *glaucoides* and *hyperboreus* at Home Bay).

The reversed-"L" $p(i)$ vs. i/d patterns observed with these 13 *Larus* populations (Fig. 2),

Fox Sparrows (*Passerella iliaca*; Zink 1986: fig. 6), Northern Flickers (*Colaptes auratus*; Grudzien et al. 1987: fig. 2), and Cory's Shearwater (*Calonectris diomedea*; Randi et al. 1989: fig. 3) are consistent with a hypothesis of high rates of gene flow. However, the reversed-"L" patterns are equally consistent with a hypothesis of shared ancestral polymorphisms among recently diverged populations, and low or no gene flow. The data sets (gulls, sparrows, flickers, and shearwaters) allow no estimation of gene flow by Slatkin's (1981) graphical approach.

Both direct estimates of *Nm* (2.1 and 10.7) suggest a fairly high gene flow among taxa, in keeping with the apparent frequency of interspecific hybridization in gulls. The discrepancy in actual estimates may be explained by the fact that the higher value, resulting from use of the "private" allele approach, lies outside the linear portion of the log *Nm* vs. log $p(1)$ relation. The robustness of this estimate is also questionable given that $p(1)$ is the average frequency of the "private" alleles, and in this case, only three alleles were found, far fewer than Slatkin's (1985) suggested minimum of 20.

Recent divergence.—The conclusion that these six species have only recently diverged and that there is high overall genetic similarity is supported by observations of phenetic similarity (Snell 1991), near genetic identity among species, shared fixed alleles at 26 of 34 loci, absence of fixed allelic differences, shared common alleles at polymorphic loci and few rare or "private" alleles. Alternatively, the 34 loci surveyed may be nonrepresentative of overall patterns of genetic variation, though there is no evidence for or against this idea. Also, it is possible that speciation was not recent, but that evolution in the nuclear genome of gulls has been constrained for unknown reasons.

Pierotti (1987) argued cogently that the specific mate-recognition systems (SMRSs) of gulls and other seabirds are exceedingly simple and probably involve the morphological cues of leg/foot coloration, bill patterning, and size. These six species of gulls are very similar phenetically, with significant differences among populations being essentially restricted to morphological size, plumage pattern, and leg/foot coloration (Snell 1991). The SMRS characters of size and leg/foot coloration evidently have a genetic basis, at least in part, as they are distinct, even in sympatry (e.g. *glaucoides* and *hyperboreus* at Home Bay, or *argentatus* and *marinus* on Kent Island). Thus, there is no reason to assume that patterns of allele frequency and divergence in the 34 surveyed loci reflect patterns of variation in the presumably few genes that control the heritable aspects of these morphological cues of the mate-recognition system.

Changes at loci that control the SMRSs may be critical to the genetic differentiation underlying the process of speciation. Patterns of genetic divergence developed over time in isolated populations (such as the accumulation of rare alleles, "private" alleles, or fixed allelic differences at electrophoretically detectable loci) may be irrelevant to the essential process of speciation.

ACKNOWLEDGMENTS

Collection of material for this study has required considerable international cooperation. The following individuals helped in collecting: Josephee Panipak, Limakee Illuak, and Hugh Graham-Marr (Home Bay); Brian Snell (Kent Island); Nelson Hurry (Prince Edward Island); Agnar Ingolfsson and Jón Halldór (Bjarnjarhafnjarfjall); J. Halldór, Skarphéthinn Thórisson and Thorhallur Borgarson (Skruder); Doreté Bloch, Geneviève Desportes, Eydfin Stefansson, and Monrad Mouretsen (Faroe Islands); Erling Norday (Svalbard); E. Norday, Karl-Birger Straum, and Robert Barrett (Tromsö); David Ashworth and John Walmsley (Camargue); and D. Ashworth, Marie-Hélène Cadiou, Ewinn Kalgariou, Jean-Paul Linard, and Michel Querné (Bretagne).

The following organizations allowed generous use of facilities: Northwest Territories Renewable Resources at Clyde River; Bowdoin College Biology Station on Kent Island; the Biology Department of the University of Iceland; the Museum of Natural History, Faroe Islands; the Institute for Arctic Research at the University of Tromsö (in Tromsö and on Svalbard); the Sysselmann, Svalbard; the Biology Department of the University of Brest, France; Centre de Recherches sur la Biologie des Populations d'Oiseaux, Brest; and the Station Biologique de la Tour du Valat, Camargue. Logistic help was provided by David Parkin (University of Nottingham) and Joe Tigullaraq (Clyde River).

Electrophoresis was done at the Laboratory of Analytical Systematics, Royal Ontario Museum. Allan J. Baker, Carol M. Edwards, Mark Peck, and Vince L. St. Louis provided advice. A. J. Baker, Jon C. Barlow, Fred Cooke, Mark Engstrom, Gillian F. Gailey, Thaddeus A. Grudzien, Roger Hansell, Tom Parsons, Jim Rising, and Kermit Ritland provided numerous criticisms of this manuscript.

This research was supported by the Natural Sciences and Engineering Research Council of Canada through a grant (A5999) to J. Rising. Additional support was received from the Canadian Wildlife Service, the Canadian Federal Ministry of Indian and Northern Affairs through the University of Toronto's Arctic Working Group, the Chapman Fund of the American Museum of Natural History, and a Sigma Xi grant-in-aid. I received an Ontario Graduate and Natural Sciences and Engineering Research Council postgraduate scholarship.

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APPENDIX. Enzymes are listed by Enzyme Commission number (International Union of Biochemistry 1984), where possible.

Polymorphic loci: 1.1.1.8 (Glycerol-3-phosphate dehydrogenase, GPD); 1.1.1.42 (Isocitrate dehydrogenase, IDH); 1.1.1.44 (6-Phosphogluconate dehydrogenase, PGD); 2.7.5.1 (Phosphoglucomutase, PGM); 3.1.1.1 (Esterase, EST-1, EST-2); 3.5.4.3 (Guanine deaminase; GDA); 5.3.1.9 (Phosphoglucose isomerase, PGI).

Monomorphic loci: 1.1.1.1 (Alcohol dehydrogenase, ADH); 1.1.1.14 (Sorbitol dehydrogenase, SDH); 1.1.1.37 (Malate dehydrogenase; MDH-1, MDH-2); 1.1.1.49 (Glucose-6-phosphate dehydrogenase, G6PDH); 1.4.1.2 (Glutamate dehydrogenase, GLUD); 1.11.1.7 (Peroxidase, PER); 1.15.1.1 (Superoxide dismutase; SOD-1, SOD-2); 2.6.1.1 (Glutamic-oxalacetic transaminase, GOT); 2.7.3.2 (Creatine kinase; CK-1, CK-2, CK-3); 2.7.4.3 (Adenylate kinase; AK-1, AK-2); 3.1.1.1 (Fluorescent esterase, Fl-EST); 3.1.3.2 (Acid phosphotase, ACP); (Erythrocyte acid phosphotase; EAP-1, EAP-2); 3.4.11 (Peptidases; PEP-A, PEP-B, PEP-C); 3.5.4.4 (Adenoside deaminase, ADA); 4.2.1.3 (Aconitase; ACON-1, ACON-2); 5.3.1.8 (Mannose phosphate isomerase, MPI).