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MOLECULAR VARIATION AND BIOGEOGRAPHY OF ROCK SHAGS[1]

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Abstract. Molecular analysis of the present genetic structure of Rock Shags indicates significant population subdivision probably caused by vicariant disjunction associated with the Llanquihue Glaciation (35,000–15,000 ybp). The formerly continuous population was forced into refugia on the Pacific and Atlantic coasts, where they remained without contact for approximately 20,000 years. With amelioration of the climate and consequent glacial retreat, populations recolonized rocky shorelines in the central portion of the present day range and introgressed considerably. The Chubút and Falkland populations serve as genetic sources for the others, whereas the Fuegian population acts as a genetic sink. The population that is resident on Isla Chiloé is enigmatic and in nonequilibrium, possibly the result of indirect effects by a yet unsampled population.

Key words: vicariance biogeography, population genetics, Fuego-Patagonia, glaciation, Phalacrocoracidae, Stictocarbo magellanicus.

INTRODUCTION

Vicariance biogeographic models postulate that allopatric remnants of a formerly continuous population are created by the interposition of a barrier to gene flow (Platnick and Nelson 1978). The most widely studied of these vicariance events have been the Pleistocene glaciations of the Northern Hemisphere (e.g., Hoffmann 1976). In typically studied cases, the range of a species was disrupted by the advancing ice sheet which displaced the population into refugia of varying sizes. Once confined to a glacial refugium, the species experienced novel conditions of habitat and species mix that presented a unique set of selection pressures, likely different for each refugium (Hoffmann 1981). Glacial retreat removed the geographical barrier and created a natural ecological experiment in which populations once isolated were then reintroduced. How isolation by vicariance may have played a role in the present genetic structure of species is a problem critical to understanding the role of biogeography in speciation events.

For southern South America, the Llanquihue Glaciation (35,000–15,000 years before present [ybp]) was the only major glaciation event of the Pleistocene (Mercer 1976); the next glacial period of similar magnitude occurred at the Pliocene-Pleistocene boundary, 1.2–1.0 million ybp. At maximum, the Llanquihue glacial sheets covered the southern Andes from approximately 40°S latitude to the Fuegian archipelago, and east to the Atlantic coast to about 52°S latitude. During this period, the Fuegian coastline (i.e., Pacific coast of southern Chile, Tierra del Fuego, and the Atlantic coast of southern Argentina) probably was uninhabitable by coastal breeding animals, because formerly-used shoreline habitats were subsequently covered by ice or drastically altered by changing sea levels (Mercer 1976, Vuilleumier 1985). Based on geological and fossil evidence, coastal refugia existed in the Falkland Islands (Islas Malvinas) as well as the adjacent Burdwood Bank that was exposed by lowered sea levels, the Atlantic coast of northern Argentine Patagonia, and in regions north of Isla Chiloé, Chile (Vuilleumier 1971). Evidence to date indicates that the fauna of patagonian Chile, patagonian Argentina, and Tierra del Fuego (i.e., Fuego-Patagonia) experienced changes as profound as those studied in the northern hemisphere (Vuilleumier 1971, 1985, Baéz and Scillato Yane 1979, Simpson 1979, Fjeldså 1985, Haffer 1985, Rasmussen 1987, 1991). In order to assess the magnitude and effect of the Llanquihue Glaciation on coastal animals, I analyzed the patterns of intraspecific genetic variation in Rock Shags (Phalacrocoracidae: *Stictocarbo*

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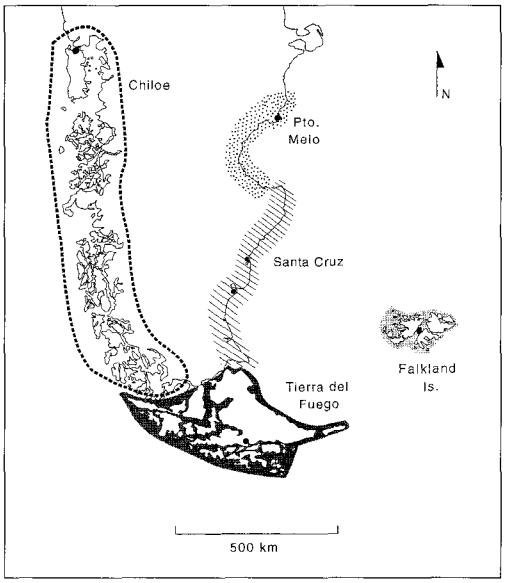

FIGURE 1. Distribution of *Stictocarbo magellanicus* in the Fuego-Patagonian regions of Argentina and Chile in southern South America. Sampled populations are: Chubút (stippling), Santa Cruz (diagonal lines), Isla Malvinas (Falkland Islands) (light shading), Tierra del Fuego (dark shading). The breeding distribution of Pacific populations is largely unknown, except for Isla Chiloé, and putative limits are indicated by dotted lines. Sampled colonies are indicated by closed circles.

magellanicus), an endemic seabird of Fuego-Patagonian marine littoral habitats (Fig. 1).

Rock Shags are primarily cliff-nesting birds feeding in shallow onshore waters and are dependent upon suitable rocky coastlines for breeding, feeding, and roosting habitats (Murphy 1936, Siegel-Causey 1986, Punta and Saravia 1993). The present distribution of Rock Shags includes the region that matches precisely the extent of the Llanquihue Glaciation plus Atlantic coastal Patagonia and the Falkland Islands; although Rock Shags are essentially a marine bird, small freshwater populations have been discovered on Lago Fagnano in Tierra del Fuego within flying distance of rocky marine shores (Chebez and Gómez 1988). Rocky habitats are patchy throughout Fuego-Patagonia and their disappearance as a result of a growing ice sheet might extirpate Rock Shags from large parts of its former range. Previous studies have indicated regional differences in plumage, morphology, and behavior, which suggests that population subdivision is important (Murphy 1936, Siegel-Causey 1986, Rasmussen 1987, Siegel-Causey and Bromley, unpubl. data).

These and other details of natural history and ecology suggest that the Llanquihue Glaciation had great impact upon the present genetic structure of Rock Shags. There are several alternative hypotheses relating to a population-level response to a glacial vicariance event in southern South America. The Llanquihue Glaciation could have forced birds onto (a) a Pacific coast refuge, (b) an Atlantic coast refuge, or (c) both Pacific and Atlantic refuges. The null hypothesis is (d) there were no refuges or dispersals associated with the Llanquihue Glaciation. Post-glaciation dispersal in hypotheses *a, b,* and *c* was bicoastal, because that is the present limits of distribution. Choosing among alternative hypotheses will require scrutiny of the gene flow patterns among the present populations of Rock Shags. For example, hypothesis *a* predicts that gene flow patterns will be unidirectional from Isla Chiloé through Tierra del Fuego to Chubút, Argentina, whereas hypothesis *b* predicts the opposite. Hypothesis *c* predicts that gene flow patterns will be centripetal and focused on Tierra del Fuego. If there is no significant pattern of gene flow, or no differences among populations, then the null hypothesis will not be falsified. In this study, I examine the geographic variation in allozymic loci in Rock Shags collected throughout the breeding range and I use various genetic measures to assess the magnitude and direction of putative gene flow among present day populations.

MATERIALS AND METHODS

Tissue samples from 90 Rock Shags were collected at coastal sites in Fuego-Patagonian Argentina and Chile from 1985 to 1987. Localities in Argentina included Puerto Melo (Chubút Province), Puerto Deseado and Monte Léon (Santa Cruz Province), and Ushuaia (Tierra del Fuego); in Chile, Challahue and Isla Chiloé (Région X). Tissues from three Rock Shags were obtained from the Falkland Islands in 1984. Morphological and natural history data (Siegel-Causey, unpubl. data), and preliminary genetic data using RFLP analysis (Bromley and Siegel-Causey, unpubl. data) indicated that birds collected from the two localities in Santa Cruz Province were not distinct, so these were analyzed together. Liver, pectoral muscle, and heart muscle were collected, frozen in liquid nitrogen within three hours of collection, and

transported by air on dry ice at liquid nitrogen temperatures, and thereafter stored in an ultracold freezer at −70°C. Tissues were homogenized by mortar and pestle at 4°C in a grinding buffer with a 1% NADP-mercaptoethanol solution and then centrifuged at 6,000× g for 5 minutes. The supernatant was stored at −70°C until used for electrophoresis. All supernatants were analyzed within 90 days of preparation.

I surveyed 39 enzyme systems comprising 65 presumptive allozymic loci. All loci were examined for all individuals at least twice. The replicate runs were designed to detect possible cryptic differences in mobility of alleles, potential post-translational modifications of allozymes caused by extended freezing and repeated freeze-thaw cycles. Presumptive loci were visualized using techniques and recipes summarized in Murphy et al. (1990). Electrophoreses were carried out on horizontal 10% starch gels or on vertical polyacrylamide slab gels (Johnson 1976, 1979). Five enzyme systems comprising seven allozyme loci (aldehyde reductase, carbonic anhydrase, malate dehydrogenase, succinate dehydrogenase, and xanthine dehydrogenase) gave unreliable, unreplicated, or ambiguous results and were not used in the analysis. Five allozyme loci (CK-2, EST-3, ICD-3, PGK-2, PGM-3) showed evidence of extensive post-translational modification, probably from repeated freeze-thaw cycles of supernatants, and also were coded as ambiguous and not used in the analysis. For the remaining 53 loci, the mobility of the most common allelic product for each locus was used as the reference and designated as allele *a* with a mobility of 100. Other alleles of a locus were labeled alphabetically beginning with the most anodal; mobilities were assessed as percent migration relative to the reference allele.

I used the BIOSYS-1 software program (Swofford and Selander 1989) for all genetic computations including allelic and genotypic frequencies, observed (direct count) and expected heterozygosities, heterogeneity chi-square values for each locus, chi-square tests for departures from Hardy-Weinberg genotypic expectations within each sample, and Wright's (1978) measures of population subdivision (*F*-statistics). The significance of deviation from Hardy-Weinberg equilibrium values was assessed using the standardized residual of individual chi-square cells. For cell (i,j), the standardized residual is defined as

$$e_{ij} = \frac{(n_{ij} - E_{ij})}{\sqrt{E_{ij}}}$$

where n_{ij} is the observed occurrence of a given genotype and E_{ij} is the expected number. An estimate of the variance of e_{ij} can be calculated as follows (Everitt 1977):

$$v_{ij} = \left(1 - \frac{n_{i+}}{n_{++}}\right)\left(1 - \frac{n_{+j}}{n_{++}}\right)$$

where n_{i+} is the row total, n_{+j} the column total, and n_{++} the grand total of observed values. With the estimate of the variance, v_{ij}, the standardized residual can be transformed to a standard normal deviate with mean of zero and unit variance. The transformation yields an adjusted residual defined as (Everitt 1977):

$$d_{ij} = \frac{e_{ij}}{\sqrt{v_{ij}}}$$

These values are distributed as a standardized normal variant z and significance and sign of deviation can be assessed directly (e.g., $|z| > 1.96$, $P < 0.05$; $|z| > 2.56$, $P < 0.01$; $|z| > 3.29$, $P < 0.001$).

I used Slatkin's (1985) formula for estimating levels of gene flow, namely:

$$\ln(p(1)) = a \ln(Nm) + b$$

To estimate direction of gene flow, I used Hedrick's (1971, 1975) *U* index, which measures the probability and asymmetry of a unique genotype relative to its absence in another population. Hedrick's *U* is calculated using the formula:

$$U_{km} = \frac{1}{L_p} \sum_{i=1}^{L_p} \sum_{j=1}^{g_i} P_{ijk}, P_{ijm} = 0$$

where i is the locus and L_p the number of polymorphic loci, j is the genotype and g_i the number of genotypes at locus i, P_{ijk} is the frequency of genotype j at locus i in locality k. U_{km} is the mean probability of drawing a genotype from region k that is not from region m. If both sets of genotypes are drawn from the same population, U_{km} and U_{mk} will be the same; if not, then unique genotypes will be distributed asymmetrically and U_{km} and U_{mk} will differ. Derivative populations will therefore have the least probability for unique genotypes and ancestral populations will have greater probability values (Hedrick 1971, 1975). The sign of the difference between two

TABLE 1. Allele frequencies of polymorphic loci for Rock Shags.

Loci[1]	Allele[2]	Mobility[3]	Falkland $n = 3$	Pto Melo $n = 19$	Santa Cruz $n = 33$	Ushuaia $n = 18$	Chiloe $n = 17$
ADA-1	a	1.00	0	0.868	0.818	0.684	0.765
	b	1.04	0.833	0.105	0.106	0	0
	c	1.11	0	0.026	0.045	0.158	0
	d	1.19	0.167	0	0.030	0.158	0.235
ALD-1	a	1.00	1.000	0.974	0.970	1.000	1.000
	b	1.06	0	0.026	0.030	0	0
EST-2	a	1.00	1.000	1.000	1.000	0.861	0.882
	b	1.17	0	0	0	0.139	0.118
G6P-1	a	1.00	0.333	0.972	0.864	0.722	0.800
	b	0.94	0.667	0.028	0.136	0.194	0.067
	c	1.11	0	0	0	0.083	0.133
GDA-1	a	1.00	0.667	1.000	0.939	0.778	0.647
	b	1.05	0	0	0	0.139	0
	c	1.17	0	0	0	0.083	0.353
	d	1.21	0.333	0	0.061	0	0
GLO-1	a	1.00	1.000	1.000	1.000	0.944	0.794
	b	0.85	0	0	0	0.056	0.206
GPD-1	a	1.00	1.000	0.895	0.894	0.357	0
	b	1.03	0	0.105	0.106	0.464	0.853
	c	1.11	0	0	0	0.179	0.147
GPI-1	a	1.00	0.333	0.842	0.848	0.917	0.765
	b	1.09	0.667	0.158	0.152	0.056	0
	c	0.94	0	0	0	0.028	0.235
ICD-1	a	1.00	1.000	0.868	1.000	1.000	1.000
	b	1.16	0	0.132	0	0	0
NP-1	a	1.00	1.000	0.895	0.924	0.889	0.588
	b	1.22	0	0.105	0.076	0.083	0
	c	1.28	0	0	0	0.028	0.412
PEP-A	a	1.00	1.000	1.000	1.000	0.944	0.882
	b	0.91	0	0	0	0.056	0.118
PEP-B	a	1.00	1.000	1.000	1.000	0.972	0.912
	b	1.13	0	0	0	0.028	0.088
PEP-C	a	1.00	0	0.842	0.879	0.139	0
	b	1.11	1.00	0.158	0.211	0.639	0.618
	c	1.19	0	0	0	0.222	0.382
PGM-1	a	1.00	1.000	0.868	0.970	0.889	0.941
	b	1.04	0	0.132	0.030	0.111	0.059

[1] Loci abbreviations follow standard usage (e.g., Murphy et al. 1990).
[2] Allele *a* is the most common form; others are listed alphabetically relative to mobility and frequency.
[3] Mobilities are relative and calculated as per cent distance of allele *a*.

populations will indicate the direction of the difference, or in other words, indicate which of the two is likely the source and which is likely the recipient of gene flow (Hedrick 1971, pers. comm.).

RESULTS

GENETIC VARIATION WITHIN POPULATION SAMPLES

Of the 53 loci scored, 14 (26.4%) were polymorphic in at least one population (Table 1) and 39 were monomorphic among all five populations (ACN-1, ACN-2, ACP-1, ACP-2, ADA-2, AK-1, ADH-1, ADH-2, ALD-2, CK-1, ENO-1, EST-1, FUM-M, FUM-S, GDH-1, GLU-1, GOT-1, GOT-2, GAPD-1, G3PD-1, GPT-1, GSR-1, HK-1, ICD-2, LDH-1, LDH-2, MPI-1, MPI-2, NP-2, PEP-D, PEP-S, PFK-1, PGD-1, PGK-1, PGM-2, SOD-1, SOD-2, SDH-1, TPI-1). The percentage of polymorphic loci ($\hat{P}$), mean number of alleles per locus ($\overline{A}$) and mean observed ($\overline{H}_o$) and expected ($\overline{H}_e$) heterozygos-

TABLE 2. Polymorphic loci ($\hat{P}$ at 0.99 criterion), mean alleles per locus ($\bar{A} \pm$ SE), pooled heterozygosities ($\bar{H}_o \pm$ SE, $\bar{H}_e \pm$ SE), Wright's F_{is}, and estimated gene flow Nm for five populations of Rock Shags.

Population	n	$\hat{P}$	$\bar{A}$ (SE)	$\bar{H}_o$ (SE)	$\bar{H}_e$ (SE)	F_{is}	Nm
Falkland	3	8.3	1.08 (0.04)	0.019 (0.007)	0.030 (0.015)	0.250	—[1]
Puerto Melo	19	17.0	1.19 (0.06)	0.019 (0.007)	0.033 (0.011)	0.340	1.60
Santa Cruz	33	15.1	1.19 (0.07)	0.021 (0.008)	0.029 (0.010)	0.221	1.54
Ushuaia	18	22.6	1.36 (0.01)	0.055 (0.016)	0.068 (0.021)	0.069	0.55
Chiloe	17	22.6	1.25 (0.07)	0.016 (0.006)	0.070 (0.019)	0.785	0.23

[1] Not available due to small sample size.

ity estimates are given in Table 2. $\hat{P}$-values ranged from 8.3% (Falkland Islands) to 22.6% (Tierra del Fuego and Isla Chiloé); the average over all individuals was 17.0%. A-values ranged from 1.08 (Falkland Islands) to 1.36 (Tierra del Fuego); the average over loci and samples was 1.19. Populations differed significantly in $\hat{P}$-values (Kolmogorov-Smirnov two-sample test, $D = 4.61$, df = 2, $\hat{P} < 0.05$), but not in $\bar{A}$-values (Kruskal-Wallis test, $P > 0.10$). $\bar{H}$-values ranged from 0.016 (Isla Chiloé) to 0.055 (Tierra del Fuego); the mean across all populations of Rock Shags was 0.039. Residual analysis of mean observed heterozygosity values indicated that all populations maintained similar levels of heterozygosity except for the Fuegian population, which had a greater than expected proportion of heterozygous genotypes ($z = 2.16$, $P = 0.031$).

The inbreeding coefficient, F_{is} (Wright 1978) was calculated for each polymorphic locus in each population. Values of F_{is} measure deviation from the expected number of heterozygous genotypes per locus and ranged from −0.161 to 1.000. The average F_{is} of the samples, calculated over all polymorphic loci, ranged from 0.069 to 0.785 (Table 2). Chi-square tests for conformity to Hardy-Weinberg equilibrium (Table 3) revealed 11 cases out of 45 for which observed and expected distribution of genotypes were significantly different ($P < 0.05$). Nine of these cases were from the Isla Chiloé population of Rock Shags. F_{is} values for these loci were high and positive, normally indicating a deficiency of heterozygotes. Residual analysis (Table 4), however, revealed that significant excess of rare homozygotes was much more important (77% of all deviating genotypic frequencies). These values imply that all populations except for the Isla Chiloé population are close to Hardy-Weinberg equilibrium. To assess the effect of results from the Isla Chiloé population, I did subsequent analyses with and without their inclusion.

TABLE 3. Chi-square values (χ^2), degrees of freedom (in parentheses), and levels of significance (corrected by strict Bonferroni criteria) for the Hardy-Weinberg equilibrium test of genetic equilibrium at each locus.

Loci	Falkland $n = 3$	Pto Melo $n = 19$	Santa Cruz $n = 33$	Ushuaia $n = 18$	Chiloe $n = 17$
ADA-1	0.01 (1)	1.03 (3)	5.22 (6)	4.28 (3)	1.06 (1)
ALD-1	M[1]	0.01 (1)	0.06 (1)	M	M
EST-2	M	M	M	0.01 (1)	0.92 (1)
G6P-1	1.28 (1)	0.01 (1)	2.38 (1)	0.05 (3)	7.81 (3)*
GDA-1	0.01 (1)	M	0.10 (1)	2.61 (3)	14.90 (1)***
GLO-1	M	M	M	0.01 (1)	8.90 (1)**
GPD-1	M	1.09 (1)	0.21 (1)	5.32 (3)	6.75 (1)**
GPI-1	1.28 (1)	4.44 (1)*	1.49 (1)	0.01 (3)	14.34 (1)***
ICD-1	M	7.66 (1)**	M	M	M
NP-1	M	1.08 (1)	1.09 (1)	0.01 (3)	8.26 (1)**
PEP-A	M	M	M	0.01 (1)	12.39 (1)***
PEP-B	M	M	M	0.01 (1)	2.47 (1)
PEP-C	M	0.11 (1)	3.68 (1)	1.25 (3)	11.25 (1)***
PGM-1	M	0.41 (1)	0.01 (1)	0.01 (1)	8.36 (1)**

[1] Monomorphic locus.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4. Genotype frequencies (and adjusted residuals) for non-equilibrium loci in Rock Shags from three populations in southern South America.

Loci	aa	ab	ac	ad	bb	bc	bd	cc	cd	dd
					Pto Melo[1]					
ADA	0.789	0.105	0.053		0.053	0		0		
	(0.19)[2]	(−0.83)	(0.11)		**(2.08)**	(−0.33)		(0.00)		
GPD	0.842	0.105			0.053					
	(0.22)	(−0.87)			**(2.08)**					
GPI	0.789		0.105					0.105		
	(0.43)		(−1.4)					**(2.50)**		
ICD	0.842	0.053			0.105					
	(0.12)	(−1.64)			**(3.33)**					
NP	0.842	0.105			0.053					
	(0.22)	(−0.87)			**(2.08)**					
PGM	0.789	0.158			0.053					
	(0.19)	(−0.69)			**(2.08)**					
					Santa Cruz					
ADA	0.697	0.091	0.091	0.061	0.061	0	0	0	0	
	(0.05)	(−1.17)	(0.32)	(0.26)	**(2.95)**	(−0.57)	(−0.46)	(−0.21)	(−0.30)	(−0.12)
NP	0.879	0.091			0.030					
	(0.16)	(−0.78)			**(2.16)**					
PEP-C	0.818	0.121			0.030					
	(0.31)	(−1.18)			**(2.39)**					
					Chiloé					
G6P	0.647	0	0.118		0.059	0		0.059		
	(0.48)	(−1.29)	(−0.72)		**(5.24)**	(−0.53)		(1.74)		
GDA	0.647		0					0.353		
	(1.51)		**(−2.83)**					**(2.83)**		
GLO	0.765	0.059			0.176					
	(0.73)	**(−1.98)**			**(2.96)**					
GPD					0.824	0.059		0.118		
					(0.14)	(−1.62)		**(3.08)**		
GPI	0.765	0			0.235					
	(1.00)	**(−2.51)**			**(3.42)**					
NP	0.529		0.118					0.353		
	(1.35)		**(−2.27)**					(1.95)		
PEP-A	0.882	0			0.118					
	(0.50)	(−1.91)			**(4.26)**					
PEP-C					0.588	0.059		0.353		
					(1.44)	**(−2.53)**		**(2.37)**		
PGM	0.941	0			0.059					
	(0.25)	(−1.39)			**(5.60)**					

[1] All loci and genotypes at Ushuaia were in equilibrium; the small number of samples from the Falkland Islands precluded analysis.
[2] Significance of genotypic deviation was assessed using the adjusted residual of a contingency chi-square test on genotypic frequencies. Sign indicates direction of deviation, bold face indicates significant deviations ($|z| > 1.96$, $P < 0.05$; $|z| > 2.56$, $P < 0.01$; $|z| > 3.29$, $P < 0.001$).

TABLE 5. Mean fixation values over all populations for polymorphic loci. Loci showing significant among-sample heterogeneities in allelic frequency distribution are indicated by asterix (*G*-tests corrected using strict Bonferroni criteria).

Locus	All localities			Excluding Isla Chiloé		
	F_{is}	F_{it}	F_{st}	F_{is}	F_{it}	F_{st}
ADA-1	0.168	0.492	0.389***	0.119	0.493	0.425***
ALD-1	−0.027	−0.005	0.021	−0.027	−0.007	0.020
EST-2	0.110	0.188	0.082	−0.161	−0.036	0.108
G6P-1	0.479	0.613	0.257**	0.422	0.580	0.273**
GDA-1	0.270	0.398	0.175*	−0.089	0.075	0.151***
GLO-1	0.607	0.657	0.128	−0.059	−0.014	0.042
GPD-1	0.520	0.763	0.505***	0.459	0.614	0.286***
GPI-1	0.694	0.776	0.268**	0.596	0.711	0.285
ICD-1	0.770	0.795	0.108	0.770	0.793	0.102*
NP-1	0.472	0.573	0.192*	0.211	0.233	0.027
PEP-A	0.645	0.668	0.065	−0.059	−0.014	0.042
PEP-B	0.468	0.495	0.052	−0.029	−0.007	0.021
PEP-C	0.471	0.736	0.500***	0.281	0.673	0.545***
PGM-1	0.260	0.289	0.039	0.296	0.322	0.037
MEAN	0.429	0.610	0.317	0.283	0.504	0.307

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

GENETIC VARIATION AMONG POPULATION SAMPLES

Three patterns predominated among continental populations of Rock Shags (Table 1). For some alleles, fixation levels increased from the Pacific side to the Atlantic side of Fuego-Patagonia (e.g., ADA-1[a], GDA-1[a], GLO-1[a], PEP-A[a]). For others, this pattern was reversed and the level of fixation increased from the Atlantic side to the Pacific side (e.g., ADA-1[d], GPD-1[b], PEP-C[c]). The third pattern was where allelic frequencies in the Fuegian population differed significantly (Kolmogorov-Smirnov two-sample test, $P < 0.05$) from neighboring populations (e.g., ADA-1[c], GDA-1[c], G6P-1[a], GPI-1[a]). Rock Shags from the Falkland Islands appeared fixed for all but four loci, but this is likely an artifact of sample size.

Only two populations showed any evidence of unique autapomorphic alleles. Rock Shags from Puerto Melo had in moderate frequency ($f = 0.132$) a fast migrating allele of ICD-1. A moderate proportion ($f = 0.139$) of birds from Tierra del Fuego were found to have an allele slightly cathodal (1.05) of the common allele of GDA-1. A much more common pattern seen in Rock Shags was synapomorphic alleles shared by adjacent populations (e.g., GDA-1[c], NP-1[c], PEP-A[b], PEP-C[c] for Tierra del Fuego and Isla Chiloé).

Overall genetic differentiation among populations was calculated for each polymorphic locus (Table 5). Population subdivision as measured by F_{st} (Wright 1978) ranged from 0.021 (ALD-1) to 0.505 (GPD-1). Across localities and loci, the significant heterozygosity chi-square ($\chi^2_{92} = 628.3$, $P < 0.001$) indicated the existence of geographic subdivision. Seven loci (ADA-1, G6P-1, GDA-1, GPD-1, GPI-1, NP-1, PEP-C) were more variable among populations than they were within a given population of Rock Shags. The mean of these F_{st} values was 0.317, a very high value for birds. When birds from the population resident on Isla Chiloé were excluded from this analysis, the mean F_{st} value (Table 5) was reduced slightly to 0.307, still well outside the limits expected for an equilibrium population of birds (Barrowclough 1983). The pattern of fixation values among loci, however, remained similar to fixation values when all populations were considered. Values were reduced further when birds from Isla Chiloé and Falkland Islands were excluded from analysis, but the mean F_{st} still was high (0.135; data not shown).

Analysis of the levels of gene flow indicated that the estimated number of immigrants per generation (*Nm*) received by the Atlantic coastal populations (Puerto Melo and Santa Cruz) was approximately the same (Table 2). By contrast, Rock Shags resident in Tierra del Fuego and Isla Chiloé had much smaller levels of gene flow (e.g., 0.55 and 0.23, respectively). The general estimate of *Nm* considering all individuals was

TABLE 6. Hedrick's probability (U_{km}) of a unique genotype averaged over 14 polymorphic loci for five localities of *Stictocarbo magellanicus*.

	Mean Probability of Drawing a Genotype That Does Not Occur In:				
From:	Pto Melo	Sta Cruz	Ushuaia	Chiloé	Falkland
Pto Melo	—	0.021	0.031	0.066	0.316
Sta Cruz	0.048	—	0.033	0.061	0.368
Ushuaia	0.181	0.153	—	0.074	0.463
Chiloé	0.162	0.103	0.059	—	0.431
Falkland	0.333	0.048	0.333	0.333	—

1.12. Jackknifed estimates of *Nm* were made by serially removing samples and assessing the effect; this process, however, did not reveal any significant outliers.

Hedrick's probabilities of a unique genotype (U_{km}) were largest for Falkland Island birds (Table 6) and to a lesser extent, Puerto Melo; a large value of U_{km} is characteristic of an ancestral region (Hedrick 1971, 1975). U_{km} also is useful in identifying regions likely to yield genotypes not found elsewhere. For example, the probability of drawing a genotype from Isla Chiloé that was not present in Puerto Melo was 0.162, but was only 0.059 compared to Tierra del Fuego. In other words, the probability that Rock Shags from Isla Chiloé and Puerto Melo were genetically distinct was more than twice that between Isla Chiloé and Fuegian populations. By contrast, a unique genotype drawn from the Santa Cruz population had a probability of 0.368 of being absent from the Falkland Islands population; the reverse situation of a unique genotype found in the Falklands Islands population but not in the Santa Cruz population was only 0.048. This indicates that the genotypes found in the Falkland Islands birds was a subset of those sampled from Santa Cruz, and that the Santa Cruz population comprised a greater diversity of genotypes.

The difference between paired U-values (i.e., $U_{net} = U_{km} - U_{mk}$) provides an estimate of the magnitude and direction of gene flow among populations (e.g., Hedrick 1971, 1975, Lubbers 1991). From the example above, the difference in U-values between Santa Cruz and Falkland Island populations was +0.320 relative to Santa Cruz, indicating a strong gene flow towards Santa Cruz from the Falkland Islands. The difference in U-values between Puerto Melo and Tierra del Fuego relative to Puerto Melo was −0.150, or a moderate gene flow from Puerto Melo towards Tierra del Fuego. These results are shown graphically in Figure 2, where directions and magnitudes are indicated by arrow. Two patterns are immediately obvious. First, some populations such as Puerto Melo act as a genetic source and some serve as a genetic sink such as Tierra del Fuego. Populations at these localities have U_{net} values with the same sign. In other words, the arrows all point the same direction: either all out, indicating flow of unique genotypes away from the population (centrifugal movement), or all in, indicating the reverse (centripetal movement). Second, some populations including those resident in the Falkland Islands, Santa Cruz, and Isla Chiloé serve both as source and recipient. In all cases, however, the net probability of finding a genotype at a given locality that is not found anywhere else is the sum of all U_{net} values for that locality (see Fig. 2). Thus, the total net probability

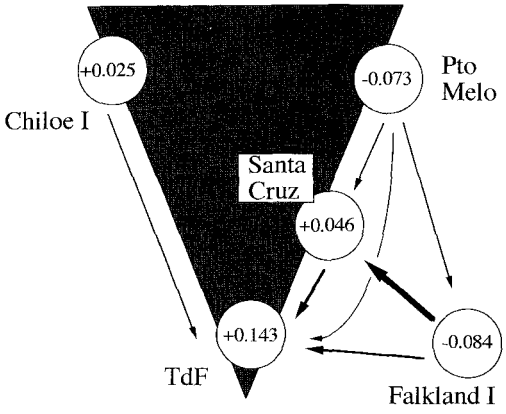

FIGURE 2. Diagram of the putative gene flows (measured by Hedrick's U-value) among populations of Rock Shags. Arrows represent magnitude of the difference between paired U-values between populations (thin lines, $|U_{net}| < 0.20$; thick lines, $|U_{net}| > 0.30$). Circles with values represent localities and sum of all U-values associated with the Rock Shag population.

of finding unique genotypes within the Falkland Islands population that were shared among other Rock Shag populations was −0.084, which indicates a centrifugal movement of genotypes primarily towards the Santa Cruz population. The total net probability of drawing a unique genotype for the Fuegian population was +0.143, indicating strong centripetal flow. By contrast, the total net probability for the Isla Chiloé population was near zero (U_{net} = 0.025), suggesting that net exchange of genotypes is static. It is tempting to apply significance values to Hedrick's probabilities, but the underlying probability distribution and statistical properties of U_{km} have yet to be determined (Hedrick, pers. comm.), so quantitative assessment of these results must await further study.

DISCUSSION

GENETIC DIVERSITY AMONG POPULATIONS

In this study of Rock Shags, the percentage of polymorphic loci ($\hat{P}$), the mean number of alleles per locus ($\overline{A}$), and observed levels of mean heterozygosity ($\overline{H}_o$) are consistent with the results of other allozyme surveys of non-passerine birds (Barrowclough 1983). Observed heterozygosity levels for all but one population were similar; the exception was found in birds resident in Tierra del Fuego. There, the proportion of heterozygotes in the population was nearly three times that found in neighboring populations and somewhat higher than the average heterozygosity reported for most birds (H = 0.053, Barrowclough 1983). In a related study on Imperial Shags (*Leucocarbo atriceps*), which are sympatric with Rock Shags, Rasmussen (1991, 1994) found a similar pattern, but of smaller magnitude. For that species, the population on Tierra del Fuego had between 1.5 and 2 times the heterozygosity found in neighboring populations.

Further evidence suggests that the Rock Shags analyzed here were not drawn from a single panmictic population. The significant deviations from genotype frequencies expected under Hardy-Weinberg equilibrium for seven loci, the highly significant chi-square values, and the high F_{st} values all indicate strong genetic structuring within this species. The non-equilibrium status of the Isla Chiloé population clearly affects these findings, but similar results obtain when this population is excluded from analysis. Mean F_{st} values over all loci and localities are quite high for this population, almost an order of magnitude greater than the average for all birds (viz., 31.79% vs. 3.5%). The estimates of gene flow are low (between 0.55 and 1.60 immigrants per generation in equilibrium populations), but it is yet unclear whether Nm values as calculated using private alleles represent current or historical patterns, or more likely, an integration of values over an unknown period of time (Slatkin 1985a, Zink et al. 1987). Nevertheless, the mean estimate of gene flow among populations is low, only 1.12 immigrants per generation.

The pattern of directional movement of genotypes within the current range of Rock Shags (Fig. 2) reveals the strongly asymmetric nature of gene flow throughout Atlantic and Pacific coasts of southern South America. On the Atlantic coast, genetic diversity is highest in the northernmost populations near Puerto Melo and decreases southward towards Tierra del Fuego and westward from the Falkland Islands towards the mainland. Moreover, high U_{km} values here are indicative of ancestral populations and support the notion that these historically non-glaciated regions served as refugia during the Llanquihue Glaciation.

The patterns are obscured by the small sample of individuals from the Falkland Islands, but evidence presented here suggests that it was a minor refugium. By contrast, the present-day northern range of Rock Shags in Chubút Province, Argentina, appears to have served as the primary Atlantic refuge during glaciation. Allelic diversity (measured by $\overline{A}$- and $\hat{P}$-values) here matches mean species values (G-test, $P >$ 0.50), and genotypic diversity (measured by U_{km}) indicates that Puerto Melo (and presumably coastal Chubút) served as a primary genetic source pool for the population complex. F_{is} values are high, something normally associated with heterozygosity deficiency (Crow and Kimura 1970, Kimura and Ohta 1971). Residual analysis of genotypes, however, indicates that F_{is} values were affected instead by excess of rare homozygotes (e.g., ADA-1^{bb}, ICD-1^{bb}, and PGM-1^{bb}). At moderate levels, this is expected in avian populations (Barrowclough et al. 1985) and it further suggests that the Puerto Melo population has always been large.

Populations resident in Santa Cruz and Tierra

del Fuego show every evidence that they were recently colonized from regions to the north and east. U_{net} values are positive and U_{km} values show significant centrifugal exchange of genotypes from Puerto Melo and Falkland Islands populations. Furthermore, it appears that the population currently resident in Tierra del Fuego has a different genetic population structure than Rock Shags in Santa Cruz. Allelic diversity is significantly elevated and observed heterozygosity levels are higher here than anywhere else; F_{is} values are low and all genotype frequencies match expected values. These findings plus the pattern of U_{km} values strongly suggest that the Fuegian population has received genetic material from northern glacial refugia on both coasts.

GENETIC DISEQUILIBRIUM WITHIN PACIFIC POPULATIONS

The status of the population on Isla Chiloé is unclear and the genetic structure here is enigmatic. Allelic diversity is high. F_{is} is very high, but heterozygosity levels and genotypic diversity are low. Heterozygote deficiency is balanced by an excess of homozygotes, and similar to the Falkland Island population, Rock Shags from Isla Chiloé lost several allelic forms found otherwise in this species. The finding that this population is in Hardy-Weinberg disequilibrium for some polymorphic loci clouds easy interpretation of these results. This unexpected finding of nonequilibrium is difficult to reconcile given that all other populations of Rock Shags are genetically stable. Although I am unable to determine the nature of the disequilibrium at this time, clues to possible destablizing forces may be found in examining how Hardy-Weinberg equilibrium might be violated.

Of the eight main assumptions of the Hardy-Weinberg model (Wright 1978, Hartl 1988), possibly three were not met by birds breeding on Isla Chiloé. The population size may have been smaller than at other localities which allowed a disproportionate effect from individual genotypes, mating may have been nonrandom and individuals were assortatively (or dissortatively) pairing, or migration may have played a greater role in this population than elsewhere. Accurate estimates of population size, or for that matter most other parameters of basic biology, are unavailable for this species; however, Rock Shags on Isla Chiloé appeared to be as abundant as in any other locality (Humphrey, pers. comm.). Furthermore, I found no evidence to suggest that assortative or disassortative mating was important at any locality. Intensive study of courtship behavior suggested that although there were slight differences in form and sequence of displays between Atlantic coastal populations, sexes did not assort by morphological types (Siegel-Causey 1986). Migration by individuals from adjacent populations is less easy to rule out, although the associated values of Slatkin's *Nm* value suggest that such migration is low (Table 2).

Residual analysis of genotype frequencies within the Chiloé population (Table 4), revealed a significant underrepresentation of heterozygotes common in the range of the species (e.g., GDA^{ac}, GPI^{ab}), but a more frequent occurrence of unique, rare genotypes (e.g., GPD^{cc}, $PEP\text{-}C^{bc}$). Moreover, Rasmussen (1987) identified several juveniles with white abdomens (i.e., derived plumage states) from Chile that are consistent with autapomorphic origin in isolation. Intermediate states were noted in birds collected in Chile and from Tierra del Fuego, but none were identified from Atlantic populations. These data suggest either low level introgression in Isla Chiloé by an unsampled population (Wahlund effect), or that birds resident on Isla Chiloé were recently established and represent the expanding front of a population located elsewhere.

Considering that the distance separating Isla Chiloé and Ushuaia is roughly the length of the west coast of the United States, the existence of breeding colonies between them is not unlikely. If the Rock Shags collected on Isla Chiloé are not representative of a discrete breeding population, but instead are at the margins of an expanding colonization front, or a zone of introgression, then the findings reported here are consistent with a population in nonequilibrium admixture (Maruyama and Fuerst 1984, 1985). If so, then the present population genetic structure of Rock Shags in Fuego-Patagonia reveals not only evidence of a past response to a widespread biogeographic vicariance event, but suggests in addition that the dynamics of change have continued.

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