POPULATION DIFFERENTIATION AND EVOLUTION AMONG THICK-BILLED (Uria Lomvia) AND COMMON MURRES (U. Aalge)

VICTORIA LOUISE BIRT-FRIESEN

POPULATION DIFFERENTIATION AND EVOLUTION AMONG THICK-BILLED (*URIA LOMVIA*) AND COMMON MURRES (*U. AALGE*)

© Victoria Louise Friesen (Birt), B.Sc., M.Sc.

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ABSTRACT

The genetic structure of animal populations determines their potential for local adaptation and speciation. Most birds are highly mobile and accordingly have little genetic structuring, but some are strongly philopatric and exhibit substantial differentiation. In the present study, population differentiation and evolution were examined within two strongly philopatric seabird species, thick-billed (*Uria lomvia*) and common (*U. aalge*) murres, using morphometrics, protein electrophoresis and nucleotide sequence analysis of amplified mitochondrial DNA (mtDNA).

Four of five morphological measurements differed significantly both among three western Atlantic colonies of thick-billed murres, and between eastern and western Atlantic colonies. Electrophoretic analysis of 18 nuclear-encoded proteins indicated little genetic differentiation either among western Atlantic colonies, or across the Atlantic. Eighteen genotypes, defined by 16 variable nucleotide sites, were found within 253 base pairs (bp) of cytochrome *b* among 239 thick-billed murres from five Atlantic and two Pacific colonies. Significant genetic differentiation was found between Atlantic and Pacific thick-billed murres, concordant with geographic separation of the populations. Little genetic differentiation was found either among western Atlantic colonies, or across the Atlantic. The apparent genetic homogeneity of Atlantic colonies is inconsistent with evidence of strong natal philopatry and phenotypic differentiation of colonies, and may result from gene flow and/or recent colonizations by large founder populations.

Common murres from Hornøya, Norway were significantly larger than those from Funk Island, Newfoundland in all of five dimensions. Ten genotypes, defined by 13 variable nucleotide sites, were found within 204 bp of cytochrome *b* among 142 common murres from four Atlantic and three Pacific colonies. Significant genetic differences were found between Atlantic and Pacific common murres, in accordance with their geographic separation. Genotype frequencies also varied clinally within the Atlantic. This cline is similar to a reported cline in the incidence of 'bridling' (a white eye ring and auricular grove) in common murres, and may have resulted from colonization of the Atlantic from two or more refugial centers following the Pleistocene glaciations.

Little differentiation was found using morphometrics or protein electrophoresis among thick-billed murres breeding at different sites within each of three colonies. However, cytochrome *b* genotype frequencies differed significantly both between two ledges in one area and among four areas at Hornøya, Norway. Differentiation in mtDNA within Hornøya is consistent both with band returns, which indicate that murres often breed on their natal ledges, and with morphological evidence, which indicates that phenotypic differentiation exists within some colonies.

Analyses of cytochrome *b* nucleotide sequences of six Atlantic alcid species, two gull species, two shorebird species and a dove revealed a preponderance of transitions over transversions, and of third position over first or second position substitutions. Saturation of transitions prevented phylogenetic analyses above the family level. Nonetheless, cladistic analysis of the alcid sequences supported previously suggested phylogenies in 1) grouping murre, razorbill (*Alca torda*) and dovekie (*Alle alle*)

sequences, 2) clustering the black guillemot sequence with the murres, razorbill and dovekie, and 3) placing the Atlantic puffin (*Fratercula arctica*) outside the other alcid sequences. The phylogenetic positions of the razorbill and dovekie relative to the murres could not be resolved.

A review of mtDNA analyses indicated that genetic differentiation of populations and species is greater in freshwater and terrestrial species than in aerial or marine animals. This agrees with general dispersal capabilities, and probably relates to long-term effective population sizes.

Key words: *Alcidae, cytochrome* b, *morphometrics, murre, protein electrophoresis, polymerase chain reaction, review*, Uria

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CHAPTER I

GENERAL INTRODUCTION

POPULATION DIFFERENTIATION AND SPECIATION

The genetic structure of animal populations determines their potential for local adaptation and speciation (Mayr 1970). Genetic structure is affected by many factors, including evolutionary history, gene flow, effective population size, mutation, selection, geographical distribution, population age, and breeding biology. One of the most important of these influences is gene flow, and population genetic differentation appears to be negatively correlated with dispersal capabilities (reviewed in Avise *et al.* 1987a). Many mammals, reptiles and fishes, especially those with low mobilities or extrinsic barriers to dispersal, exhibit pronounced genetic structuring (e.g. bluegill sunfish *Lepomis macrochirus*, Avise *et al.* 1984a; salamanders, Larson *et al.* 1984; deer mice *Peromyscus maniculatus*, Ashley and Wills 1987), whereas those with greater dispersal capabilities are generally more homogeneous (e.g. American eels *Anguilla rostrata*, Avise *et al.* 1986; domestic mice *Mus domesticus*, Ferris *et al.* 1983; reviewed in Avise *et al.* 1987a).

Most birds are highly mobile and so have potential for extensive gene flow. Correspondingly, most avian populations exhibit little if any genetic structuring (reviewed in Barrowclough 1983, Evans 1987, Barrowclough and Johnson 1988; e.g. red-winged blackbirds *Agelaius phoeniceus*, Ball *et al.* 1988). However, many birds exhibit natal philopatry (i.e. they return to natal areas to breed), and strong natal philopatry, especially if coupled with local variation in selection pressures, may result in genetic differentiation

of demes despite high dispersal abilities. Accordingly, genetic structuring is substantial within some avian species. For example, many waterfowl and seabirds are strongly philopatric, and significant genetic structuring has been found among Canada geese (*Branta canadensis*; Van Wagner and Baker 1990, Shields and Wilson 1987a), black brant (*B. bernicla*; Shields 1990), Cory's shearwaters (*Calonectris diomedea*, Randi *et al.* 1989) and fairy prions (*Pachyptila turtur*, Ovenden *et al.* 1991).

Understanding of population genetic structure is theoretically important since the evolution of species and higher taxa was considered classically to involve a temporal extension of processes of population differentiation (Mayr 1970). However, these processes do not satisfactorally explain all cases of speciation, and modern researchers sometimes invoke other explanations. For example, most avian populations studied to date exhibit little genetic structure, but rates of morphological evolution and speciation appear to be higher in birds than in most vertebrates (Wyles *et al.* 1983). Wyles *et al.* (1983) therefore suggested that culturally transmitted behaviour can provide a driving force ('behavioural drive') for morphological specialization and speciation (see also Hafner *et al.* 1984, Wilson *et al.* 1984). As another example, Carson and Templeton (1984) proposed founder induced genetic revolutions to account for the plethora of Hawaiian *Drosophila* species.

Population genetic structure also is a focal concern for conservation biology. If populations differ genetically, then loss or decimation of a deme can reduce a species' genetic resources, and thus its longterm viability (Allendorf and Leary 1986, Gilpin and Soulé 1986). Genetic differentiation is also inversely correlated with gene flow, and

recolonization of depopulated areas by geographically differentiated species may be slow or nil. Such species may require protection over most of their range. Conversely, animals with little or no population genetic differentiation may be more resilient to local extinctions or decimations, and protection of large parts of their range may be less critical.

THE MURRES

Thick-billed and common murres (*Uria lomvia* and *U. aalge*, respectively) are cold-water seabirds that may migrate hundreds to thousands of miles annually between breeding colonies and wintering grounds (Gaston 1980, Brown 1985a, Kampp 1988), and may travel hundreds of miles daily between nesting and feeding sites (e.g. Gaston 1985a, Cairns *et al.* 1987). Nonetheless, band returns indicate that murres are strongly philopatric (Birkhead 1977, Kampp 1988, Noble *et al.* 1991), and morphometric differences among colonies suggest that colonies may differ genetically (Storer 1952, Mayr 1970, Birkhead and Nettleship 1981, Gaston *et al.* 1984, Boag and van Noordwijk 1987).

The two species of murres are very similar in most biological aspects, and are virtually identical in biological parameters that affect population genetic structure (Tuck 1960, Nettleship and Birkhead 1985). Both have circumpolar distributions and their world populations are similar in size (4.9—7.5 million pairs of thick-billed murres *vs.* 3.0—4.5 million pairs of common murres in the Atlantic, Nettleship and Evans 1985; ~5 million pairs each in the Pacific, J.F. Piatt, *pers. comm.*). Both breed in large colonies (tens to hundreds of thousands of pairs), although colonies of common murres tend to be smaller.

Adults are monogamous and will retain mates for many years, and breeding success (fledglings/egg, ~70% in both species), survival to first reproduction (17—41% in common murres, 19—53% in thick-billed murres), age of first breeding (4—5 years each) and adult survivorship (87—90% in common murres, 91% in thick-billed murres) are similar (reviewed in Harris and Birkhead 1985, Hudson 1985). Nonetheless, sufficient morphological differences exist among Atlantic colonies of common murres to result in taxonomic subdivision of this population into between two and five subspecies, whereas Atlantic thick-billed murres are regarded as a single monotypic subspecies (*lomvia*; reviewed in Tuck 1960, Bédard 1985). The contrasting patterns of morphological differentiation between the species suggest that their population genetic structures may also differ.

Population genetic information is also required for conservation policies for murres. The Atlantic populations of both species are presently declining (e.g. Nettleship and Evans 1985, Evans and Kampp 1991). Murres of both species wintering off Newfoundland, Labrador and Greenland are the object of an annual hunt, which is both culturally and economically important to coastal communities (Tuck 1960, Evans and Waterston 1976, Montevecchi and Tuck 1987). An estimated 300,000—725,000 murres, mostly thick-billed, are shot annually during winter off Newfoundland (Elliot *et al.* 1991). Large numbers of murres are also shot near breeding colonies during summer in Arctic Canada and Greenland (Evans and Waterston 1976, Gaston *et al.* 1985, Kampp 1991). Due to their aquatic habits and marine diets, murres are also highly vulnerable to oil contamination, and many are killed each year by oil spills and chronic low-level releases

from commercial ships (Piatt *et al.* 1985). In addition, many murres drown in gill nets in eastern Canada and Greenland (Evans and Waterston 1976, Nettleship and Evans 1985, Falk and Durinck 1991; see also DeGange and Day 1991), and large numbers of eggs are taken from some breeding colonies each year (Nettleship and Evans 1985, V.L.F. *pers. obs.*). The combined annual mortality of murres, especially thick-billed, from hunting, oil, egging and gill nets in eastern Canada and Greenland appears to exceed the annual production of young, and many colonies in the western Atlantic have declined by at least 20—30% since the 1950s (Nettleship and Evans 1985, Evans and Kampp 1991). The development of oil fields in the Canadian Arctic and on the Grand Bank of Newfoundland pose an additional, direct threat. If colonies differ genetically, then protection of representative colonies from throughout the Atlantic will be crucial.

MOLECULAR ANALYSIS OF POPULATION STRUCTURE

Although protein electrophoresis provides genetic information for many nuclear loci, it detects only those differences that produce electrophoretic changes; silent nucleotide substitutions, and amino acid substitutions that do not alter a protein's electrophoretic mobility are not detected by conventional methods. The method seldom uncovers significant genetic differentiation among local populations of birds (Barrowclough 1983, Evans 1987, Barrowclough and Johnson 1988). Murre colonies generally occur within areas that were glaciated during the Pleistocene, so are probably less than 10,000 years old (although refugial colonies may have existed near polynyas, or ice-free areas within the Arctic). Genetic subdivision may therefore be too recent to

be detected using traditional protein electrophoretic techniques.

Because mitochondrial DNA (mtDNA) is haploid, non-recombining and maternally inherited, its effective population size is expected to be 1/4 that of nuclear genes. MtDNA therefore is affected by founder effects, population bottlenecks and genetic isolation much more than are nuclear genes (Birky *et al.* 1983, Wilson *et al.* 1985a). Furthermore, mtDNA evolves 5--10 times more quickly on average than do single-copy nuclear genes (Brown *et al.* 1979, Wilson *et al.* 1985a), so restriction endonuclease (RFLP) analysis of mtDNA has often proven useful for detecting population subdivision in many animals, particularly birds (e.g. Zink 1991; see also Quinn and White 1987). However, restriction analyses miss many mutations, such as restriction sites that are close together or multiple substitutions within single sites (Aquadro and Greenberg 1983, Carr and Marshall 1991, T.P. Birt *unpubl. data*).

The polymerase chain reaction (PCR), or DNA amplification, provides a powerful new tool with potential applications that span fields from forensic science through medicine to theoretical biology (Saiki *et al.* 1988; e.g. Kocher *et al.* 1989, Pääbo 1989, Bartlett and Davidson 1991). PCR involves synthesis of millions of copies of a chosen DNA segme from as few as one template copy. Basically, short pieces of single-stranded DNA ('primers' typically 20—30 base pairs [bp] long), complementary to sequences flanking a segment of interest, are combined with a tiny amount of template DNA. A thermostable DNA polymerase and the four nucleoside triphosphates are added. The mixture is heated to denature the template DNA, cooled to allow annealling of primers to template strands, then heated slightly to promote replication of the target

segment by primer extension. This temperature cycle produces two copies of the chosen segment from each template copy. If the cycle is repeated, the number of copies is again doubled (although the reaction is not 100% efficient due to such factors as reannealing between DNA strands and enzyme kinetics). Repetition of the cycle 30 to 40 times produces up to a billion copies of the target segment. The DNA product is sufficiently pure for applications such as cloning, RFLP analysis and nucleotide sequencing.

Because nucleotide sequence analysis of amplified DNA enables the primary nucleotide sequences of specific genes to be compared among individuals, populations or species, it has great potential for population genetics and systematics. It has several advantages over conventional population genetics techniques. All nucleotide sequence differences within a given region are detected; this circumvents many assumptions necessary for calculations of genetic divergence using RFLP or protein data, and increases analytical power over conventional tools. The analytical sensitivity can be adjusted by changing the target segment: regions with high evolutionary rates, such as the mitochondrial D-loop, may be compared among recently diverged populations (e.g. Vigilant *et al.* 1989), whereas highly conserved segments, such as genes for nuclear-encoded proteins or ribosomal RNAs, may be used for higher level phylogenetics (e.g. Birt *et al.* 1992). Sequence analyses provide direct insight into the evolution not only of populations and species, but also of the genetic material. Finally, field protocols are simpler than for either protein electrophoresis or RFLP analyses, and laboratory procedures are simpler than for RFLP analyses.

OBJECTIVES

In the present study, morphometrics, protein electrophoresis and direct sequence analysis of amplified mtDNA were used to investigate population differentiation and evolution within the Holarctic populations of thick-billed and common murres. Three levels of analysis were involved: 1) phenotypic and genetic differentiation within colonies of thick-billed murres (Chapter IV), 2) phenotypic and genetic structuring among colonies of thick-billed (Chapter II) and common murres (Chapter III), and 3) phylogenetic relationships among Atlantic alcids (Chapter V). Because the present study measures population genetic differentiation within two closely related species, it provides insight into mechanisms of avian population differentiation and speciation. It also provides population genetic information critical to conservation policies for two declining seabird populations.

CHAPTER II

GENETIC STRUCTURE OF THICK-BILLED MURRES

INTRODUCTION

Thick-billed murres (*Uria lomvia*) are distributed continuously throughout the Arctic, with one subspecies (*U. l. arra)* described from the Pacific, two from the Arctic (*U. l. eleonorae* from Siberia and *U. l. heckeri* from the Chukchi Sea) and one from the Atlantic (*U. l. lomvia*, reviewed in Tuck 1960, Bédard 1985). Although thick-billed murres may migrate thousands of kilometers annually between nesting colonies and wintering grounds (Gaston 1980, Brown 1985a, Kampp 1988), all of more than 600 murres that were banded as chicks and subsequently sighted as adults during the breeding season were recorded in or near their natal colonies (Noble *et al.* 1991, A.J. Gaston *unpubl. data*; see also Kampp 1988). In addition, Storer (1952) reported a north-south cline in wing length, bill length and bill depth among western Atlantic colonies, and Birkhead and Nettleship (1981) and Gaston *et al.* (1984) reported significant differences in mass, wing length and bill dimensions among thick-billed murres from neighbouring Canadian colonies. These data suggest that colonies may be genetically isolated.

In the present chapter, morphometrics, protein electrophoresis and direct sequence analysis of part of the mitochondrial cytochrome *b* gene were used to examine phenotypic and genetic relationships among thick-billed murres from five Atlantic and two Pacific colonies. Genetic isolation was predicted to produce genetic differences 1) between

Atlantic and Pacific subspecies of thick-billed murres, 2) between eastern and western Atlantic populations, and 3) among western Atlantic colonies.

METHODS

SAMPLING

Thick-billed murres were sampled from four colonies in the northwestern Atlantic (Coats, Akpatok and Coburg Islands in northeastern Canada, and Kipako in western Greenland), one in the northeastern Atlantic (Hornøya in northern Norway) and two in the north Pacific (Semidi and Buldir Islands, Alaska; Table 2.1; Fig. 2.1). At all Atlantic colonies except Coburg Island, adult murres were noosed on breeding ledges, several morphological measurements were made (see below) and ~1 mL blood was taken from a brachial vein. Because band returns indicate that murres often return to natal cliff ledges to breed (Noble *et al.* 1991; Chapter IV), adults were collected from several sites within each colony to obtain a broad representation of intra-colony variation (Table 2.1). At Coburg Island, blood was taken from nestlings caught by hand in one area only. Blood collected at Kipako was added to 14 mL of lysis buffer containing 100mM Tris-HCl (pH=8.0), 100mM EDTA, 10mM NaCl and 0.5% SDS (Longmire *et al.* 1988), and stored at ambient temperatures. All other samples were collected into Vacutainers^{R} and stored at —70°C after return to the laboratory. Muscle samples were also obtained from murres that were banded as adults or chicks at colonies and subsequently shot off Newfoundland during the winter murre hunt. Samples from the Pacific consisted of liver from murres shot near breeding colonies.

TABLE 2.1: Colony locations and sizes, and dates and sites of sample collections for thick-billed murres.

Colony (Location)	Colony Size (pairs)	Sampling Dates	Sampling Sites	Sample Size
Coats Island (62°57'N, 82°00'W)	24,000[1]	07.87	Site S	8
			Site D	4
			Site L	7
			Other breeding adults	6
		08.90	Site T	5
			Site Z	
			Ledge 1	3
			Ledge 2	6
			Ledge 3	6
		87–89	Winter murre hunt	20
			Total	65
Coburg Island (75°48'N, 79°25'W)	160,000[2]	07.87	Fledglings	20
			Breeding adult	1
		87—89	Winter murre hunt	3
			Total	24
Akpatok Island (North Colony) (60°32'N, 68°30'W)	400,000[2]	07.87	Breeding adults	30
Kipako (73°42'N, 56°35'W)	18,000[3]	07.89	Southeast corner	
			Top ledge	7
			Bottom ledge	5
			Northeast corner	8
			North end	5
			West end	2
			Other breeding adults	4
			Total	31

Table 2.1, Cont'd.

Colony (Location)	Colony Size (pairs)	Sampling Dates	Sampling Sites	Sample Size
Hornøya (72°22'N, 31°10'E)	430[4]	07.89 and 07.90	Area 1	6
			Area 2	
			Ledge U, End W	16
			Ledge U, End E	16
			Ledge B	15
			Area 3	16
			Area 4	16
			Total	85
Semidi Islands (56°00'N,156°45'W)	100,000[5]	05.90 07.90	Offshore	2
Buldir Island (52°20'N,175°55'E)	23,000[6]	05.90 06.90	Offshore	2

1) Gaston *et al.* (1987).
2) G. Chapdelaine (Canadian Wildlife Service, Ste-Foy, *pers. comm.*).
3) J. Durinck (Ornis Consult, Copenhagen, *pers. comm.*).
4) R.T. Barrett (University of Tromsø, *pers. comm.*).
5) Individuals counted on cliffs; Hatch and Hatch (1990).
6) Individuals counted on cliffs; Sowls *et al.* (1978).

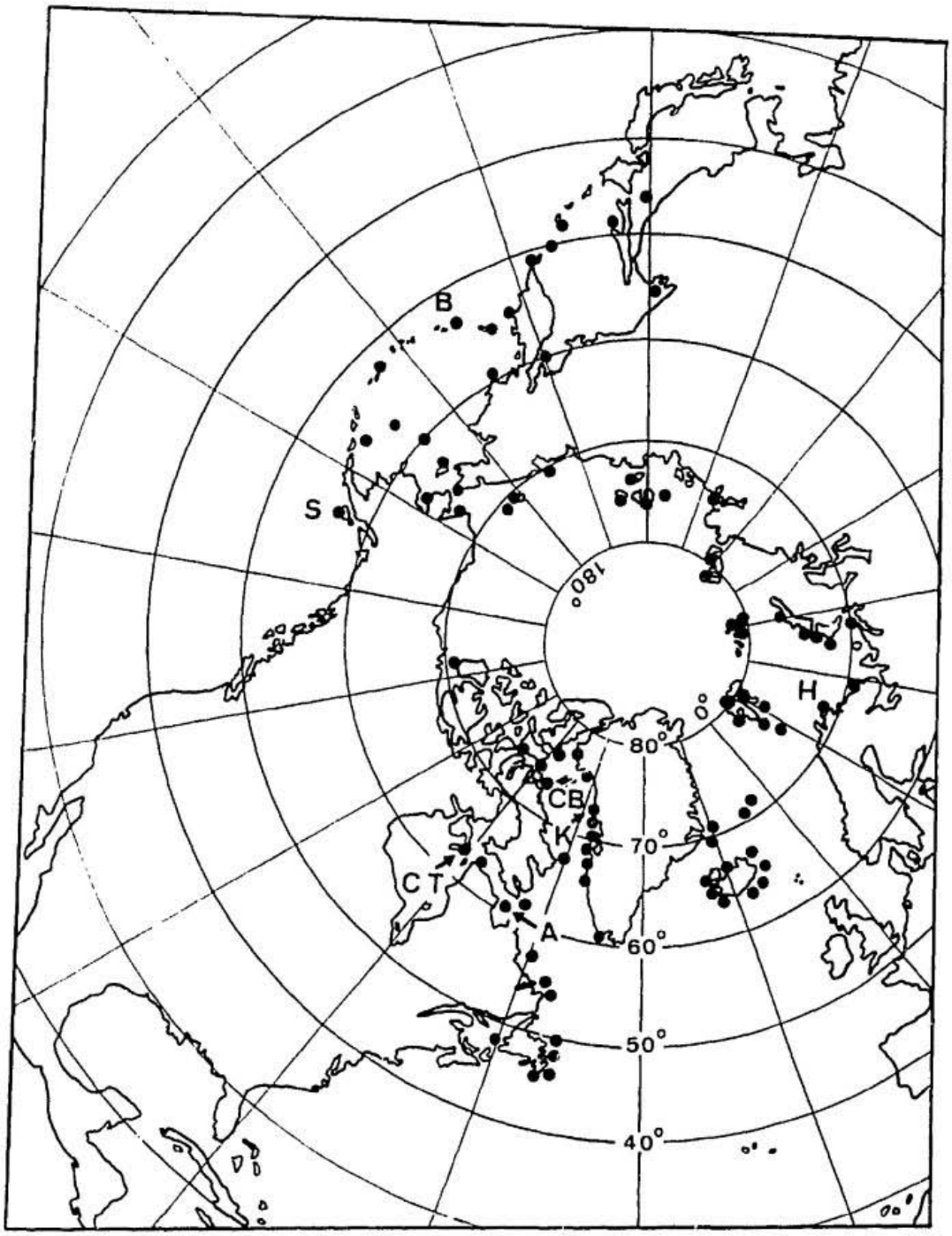


FIG. 2.1: Thick-billed murre breeding range. Dots represent colonies. A = Akpatok Island, B = Buldir Island, CB = Coburg Island, CT = Coats Island, H = Hornøya, K = Kipako and S = Semidi Islands. Redrawn from Tuck (1960).

MORPHOMETRICS

Morphological measurements were made on murres captured for blood sampling at most colonies (Table 2.2). Measurements included mass, wing length (flattened chord), culmen length (culmen to bill tip), bill depth (depth at gonys perpendicular to cutting edge), nostril (distal end of nostril to bill tip) and head+bill length (back of skull to bill tip). Masses were measured to the nearest 10g using spring balances; wings were measured to the nearest 1mm using a stopped wing-rule; bill measurements were made to the nearest 0.1mm with vernier calipers; and head+bill lengths were measured to the nearest 1mm using 'head calipers' (Coulson *et al.* 1983).

Because consistent measurement biases often exist among researchers (e.g. Barrett *et al.* 1989, V.L.F. *unpubl. data*), and because several researchers were involved in the present collections (Table 2.2), each researcher also measured 10 thick-billed murre skins obtained from the Royal Ontario Museum (Toronto, Ontario) to derive correction factors (Table 2.3). A.J. Gaston's bill depth measurements were slightly smaller than R.T. Barrett's (subject x treatment ANOVA, unbalanced design, $F_{1,9} = 5.76$, $P = 0.04$); bill depth measurements from Coats Island were therefore increased by the mean difference between A.J. Gaston's and R.T. Barrett's skins measurements (+0.11mm). No other differences were found among these researchers ($P > 0.10$). J. Durinck's measurements at Kipako were adjusted by the mean difference between his and R.T. Barrett's measurements on eight dead and eight live auks (wing length, —2mm; culmen length, —0.2mm; head+bill length +0.1mm; Barrett *et al.* 1989). Measurements made by G. Chapdelaine at Akpatok were corrected by the mean differences between his and A.J.

TABLE 2.2: Morphological measurements made on thick-billed murres at four Atlantic colonies. See text for measurement definitions.

Colony	Year	Measurements	Measurer[1]
Coats Island	1987	Mass, Wing	RDE
	1990	Mass, Wing, Culmen, Depth, Nostril	AJG
Akpatok Island	1987	Mass, Wing, Culmen, Nostril	GC
Kipako	1989	Mass, Wing, Culmen, Depth, Head	JD
Hornøya	1989 & 1990	Mass, Wing, Culmen, Depth, Head	RTB

1) RDE = R.D. Elliot, AJG = A.J. Gaston, GC = G. Chapdelaine, JD = J. Durinck, RTB = R.T. Barrett.

TABLE 2.3: Mean (±SD) measurements made by three researchers on 10 thick-billed murre skins. All measurements are in mm.

Measurer[1]	Wing	Culmen	Depth
AJG	209±6.2	13.9±0.45	33.4±1.65
RDE	209±6.9		
RTB	210±5.6	14.0±0.55	33.2±1.78

1) AJG = A.J. Gaston; RDE = R.D. Elliot; RTB = R.T. Barrett.

Gaston's measurements on 40 dead thick-billed murres (wing length, +4mm; nostril +0.4 mm; Gaston *et al.* 1984). Although corrections obtained on skins and freshly killed birds may not be identical to those for live birds, Barrett *et al.* (1989) reported no consistent differences in measurements made by 4 researchers on live *vs.* recently killed auks. No effort was made to correct for morphological differences between sexes.

Adjusted measurements were compared among colonies by analysis of variance (unbalanced design) using Bonferonni corrections for number of comparisons. The among-population component of morphological variation (a morphological equivalent to Wright's [1965, 1978] among-population component of genetic variation, F_{ST}) was calculated from the ratio of the sum of squares for treatments (populations) to the total sum of squares, averaged across measurements:

$$F_{ST} = 1/n \sum_{i} (SSP_i / SST_i)$$

where n is the number of measurements, SSP_i is the sum of squares for populations for measurement i and SST_i is the total sum of squares for measurement i (Barrowclough 1991).

PROTEIN ELECTROPHORESIS

Protein products corresponding to 20 presumptive nuclear loci could be reliably resolved from blood samples on cellulose acetate (Richardson *et al.* 1986; Table 2.4). Electromorphs for a given protein were assumed to represent alleles for the corresponding locus. Samples collected at Hornøya in 1989 were scored for all 20 proteins (Table 2.4); those from Coats, Coburg and Akpatok Islands were not scored for ALD or G6PD due to sample degradation. Samples collected at Hornøya in 1990 were scored for two

TABLE 2.4: Nuclear proteins resolved in thick-billed murres, and electrophoretic conditions employed. E.C. No. = enzyme commission numbers.

Protein	E.C. No.	Abbrev.	Running Buffer[1]	Running Time (min)
Adenosine Deaminase	3.5.4.4	ADA	T 7.4	20
Adenylate Kinase	2.7.4.3	AK	T 7.4	20
Albumin	-	ALB	TG 8.5	35[2]
Aldolase	4.1.2.13	ALD	CT 7.5	20
Erythrocyte Acid Phosphatase	3.1.3.2	EAP	TBE 8.8	30
Esterase	3.1.1.1	EST	TBE 9.1	15
Esterase D	3.1.1.1	ESTD	TBE 9.1	10[3]
Glucose-6-Phosphate Dehydrogenase	1.1.1.49	G6PD	TBE 8.8	30[4]
Glucose-Phosphate Isomerase	5.3.1.9	GPI	CT 7.5	40[2]
Haemoglobin	-	HB	TBE 9.1	40
Isocitrate Dehydrogenase	1.1.1.42	IDH	CT 7.5	40
Lactate Dehydrogenase	1.1.1.27	LDH1	T 7.4	20
Malate Dehydrogenase	1.1.1.37	MDH1	CT 7.5	40
		MDH2	CT 7.5	40
Mannose-Phosphate Dehydrogenase	5.3.1.8	MPI	CT 7.5	40
Peptidase A	3.4.11/13	PEPA	TBE 8.8	10
Peptidase B	3.4.11/13	PEPB	CT 7.5	40[4]
6-Phosphogulconate Dehydrogenase	1.1.1.44	6PGD	TBE 9.1	40
Phosphoglucomutase	2.7.5.1	PGM	TBE 8.8	30
Transferrin	-	TF	TG 8.5	35[2]

TABLE 2.4, Cont'd.

1) T 7.4 = 100mM Tris, 100mM NaH_2PO_4, pH=7.4 (Harris and Hopkinson 1976);
   TG 8.5 = 25mM Tris, 192mM glycine pH=8.5 (Richardson *et al.* 1986);
   CT 7.5 = 40mM citric acid, titrated to pH=6.0 with N-(3-aminopropyl)-morpholine and to pH=7.5 with 1,3-bis-dimethylamino-2-propanol (Clayton and Tretiak 1972);
   TBE 8.8 = 130mM Tris, 2.2mM Na_2EDTA, 6mM NaOH, 71.3mM boric acid, pH=8.8 (Richardson *et al.* 1986);
   TBE 9.1 = 130mM Tris, 2.2mM Na_2EDTA, 6mM NaOH, 71.3mM boric acid, pH=9.1 (Richardson *et al.* 1986).
2) Blood diluted 1:10 with water.
3) 5µL sample loaded.
4) 0.5µL blood incubated 10—15min at 37°C with 0.5µL freshly prepared buffer containing 100mM Tris, 10mM dithiothreitol, pH=6.0.

variable proteins only (ALB and 6PGD). Protein electrophoresis was not conducted on samples collected at Kipako due to the collection buffer.

Electrophoretic data were analyzed using the computer package BIOSYS-1 (Swofford and Selander 1981). Genotype frequencies were compared with Hardy-Weinberg expectations using contingency X^2 statistics (with and without pooling of cells). The extent of genetic differentiation among colonies was determined by 1) comparison of allele frequencies among colonies using X^2 analysis (with pooling of rare alleles), 2) calculation of the amount of genetic variation due to population subdivision (Wright's F_{ST}; Wright 1965, 1978, Kirby 1975), and 3) calculation of Nei's (1978) and Roger's (1972) genetic distances.

ANALYSIS OF THE CYTOCHROME *B* GENE

DNA was extracted from tissue samples by digesting 10—20μL blood or ~10mg muscle with 50μg protease K at 50—60°C for at least 6 hr in 750μL of 100mM Tris-HCl (pH=8.0), 10mM ETDA, 100mM NaCl and 0.1% SDS. Proteinase K was added directly to the storage buffer for blood collected at Kipako. Digested proteins were extracted twice with an equal volume of phenol (saturated with 100mM Tris-HCl, pH=8.0) and once with an equal volume of chloroform:isoamyl alcohol (24:1).

A 307 bp (376 bp including the priming sites) region of the mitochondrial cytochrome *b* gene was amplified using the polymerase chain reaction and primers L14841 (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3') and H15149 (5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3') from Kocher *et al.* (1989). This segment was chosen because the primers had proven useful in a wide variety of

vertebrates, and because its variability approximates, or slightly exceeds, the mean for the mitochondrial genome (Ferris *et al.* 1983, Wayne *et al.* 1990, Edwards and Wilson 1990, Irwin *et al.* 1991, Quinn *et al.* 1991, T.P. Birt *unpubl. data*). It had also been used successfully to differentiate populations of deer (Carr and Hughes 1992, Hughes and Carr 1992). Double-stranded product was obtained by amplification of a 1/10—1/100 dilution of DNA in 25µL of a solution of 67mM Tris-Cl (pH=8.8), 2mM $MgCl_2$, each dNTP at 0.2mM, each primer at 0.4µM and 0.5 units AmpliTaq DNA polymerase (Perkin-Elmer/Cetus). Thirty cycles of PCR were conducted in a Perkin-Elmer/Cetus thermocycler. Each cycle involved denaturation of DNA at 94°C for 30s, annealling of primers to template DNA at 55°C for 30s, and extension of primers at 72°C for 60s.

Ten µL of the reaction mixture were subjected to electrophoresis in 2% NuSieve (FMC) agarose gels containing 1µg/mL ethidium bromide, 89mM Tris-borate and 2mM EDTA (pH=8.3). A gel plug containing the double-stranded product was melted in 100µL water at 70°C for 10min, and 2µL were used as template in the generation of single-stranded DNA. Single-stranded amplifications were conducted under reaction conditions similar to those for double-stranded amplifications except that buffer volume totalled 100µL, one primer was diluted 1:100, 1 unit AmpliTaq was used, and 40 cycles of PCR were conducted. Either of two methods was used to remove buffer salts and unincorporated primer from single-stranded product and to concentrate the DNA: 1) three cycles of ultrafiltration were conducted in Centricon-30 microconcentrators (Amicon); 2) DNA was precipitated with 1 volume 4M ammonium acetate and 2 volumes isopropanol, washed with 500µL 70% ethanol (—20°C), dessicated and resuspended in 15—50µL H_2O.

Seven µL of purified single-stranded product were sequenced with the primer that was limiting in the single-stranded amplification, using α-^{35}S-dATP and the dideoxy chain termination method (Sanger *et al.* 1977; Sequenase®, United States Biochemical).

For most murres, only the heavy strand was sequenced. However, because replication errors may be introduced during amplification (e.g. Dunning *et al.* 1988, Tindall and Kunkel 1988), DNA from birds with rare genotypes (N=13) was amplified and sequenced twice for confirmation. An additional 25 samples were amplified and sequenced twice to determine reproducibility.

Nucleotide sequences were aligned and translated using the ESEE computer program (Cabot and Beckenbach 1989). Cytochrome *b* genotype frequencies were compared among colonies using Chi-square (X^2) tests. Because many expected cell frequencies were less than 5, a computerized randomization test was run (Roff and Bentzen 1989). For each randomization, a X^2 value was calculated for an artificial data set in which individuals were randomly assigned to genotypes and colonies, with totals for each genotype and colony (column and row totals) kept constant. The probability that the observed value of X^2 exceeded the X^2 values for 1000 randomizations was determined.

Interdeme genetic variation (G_{ST}) was calculated using the equation

$$G_{ST} = (H_T - \overline{H}_0) / H_T$$

where $\overline{H}_0$ is mean genetic diversity over all subpopulations, calculated as

$$H_0 = n \ (1 - \sum_i x_i^2) / (n - 1)$$

n is the number of birds sampled, and x_i is the proportion of birds with genotype i. The total or species diversity, H_T, was calculated as

$$H_T = 1 - \sum_i y_i^2$$

where y_i is the mean frequency of the ith genotype across all subpopulations (Takahata and Palumbi 1985, Rand and Harrison 1989, Ovenden and White 1990). Because estimates of G_{ST} tend to decline as numbers of genotypes and samples increase (Slatkin and Maddison 1989, Lynch 1991, V.L.F. *pers. obs.*), population subdivision was also determined using the equation

$$\gamma = 1 - (I_B/I_W)$$

where I_B is the mean over all colony pairs of $\sum_i x_r x_{r+l}$ and I_W is the mean over all populations of $\sum_i x_i^2$ (Latter 1973, Lynch 1991).

Percent sequence divergence within populations was calculated as

$$\pi = \sum_{ij} x_{ij} p_{ij}$$

where x_{ij} is the product of the frequencies of genotypes i and j, and p_{ij} is percent sequence divergence (p) between genotypes i and j (Nei and Li 1979). Genetic distance between populations was calculated as

$$D = \pi_{XY} - 0.5\ (\pi_X + \pi_Y)$$

where π_{XY} is percent sequence divergence between populations X and Y, and π_X and π_Y are percent sequence divergence within X and Y respectively (Nei and Li 1979).

Slatkin (1989) and Slatkin and Maddison (1989) argued that more information is contained in genotype geneologies than in frequency data alone. Substitutional relationships among genotypes were therefore also compared among populations.

All means are reported ± 1 standard deviation. A significance level (α) of 0.05 was used for all statistical tests.

RESULTS AND DISCUSSION

MORPHOMETRICS

Analysis of variance revealed significant differences in mass, wing length, culmen length and head+bill length between thick-billed murres from eastern and western Atlantic colonies, with those from Hornøya being larger (Table 2.5). No differences were found in nostril. The proportion of morphological variation distributed among populations (Barrowclough's [1991] $\overline{F}_{ST}$) averaged 0.18±0.14, (n = 5 measurements; Table 2.5).

Murres from the three western Atlantic colonies also differed significantly in mass, wing, culmen length and bill width (Table 2.5). Birds from Akpatok were smaller than those from the other two colonies in mass, wing length and culmen length, and murres from Kipako were larger than those from Coats Island in bill depth. F_{ST} averaged 0.18±0.096 (n = 5 measurements; Table 2.5). No differences occurred in bill depth.

These results should be interpreted cautiously, since corrections derived from skins may not adequately control for measurer differences on live birds. Mass is also highly subject to environmental variation, and often differs within individuals among years and seasons (e.g. Croll *et al.* 1991). Furthermore, measurements were not independent, so estimates of $\overline{F}_{ST}$ may be inflated. Nevertheless, in the present data set, most correction factors made inter-colony comparisons more conservative, and comparisons did not lose their statistical significance if corrections were removed. Also, colony differences in wing and bill lengths, which are less subject to environmental variation than is mass, were large and highly significant. These results are similar to those of Gaston *et al.* (1984), who reported that breeding murres from Akpatok Island had smaller masses, shorter wings and

TABLE 2.5: Means (±SD) of six morphological measurements for thick-billed murres at four Atlantic colonies, results of ANOVAs (unbalanced design), and F_{ST}s. Masses are in g; all other measurements are in mm. Sample sizes are in parentheses. Means with different letter superscripts are significantly different according to Tukey's studentized range test.

Colony	Mass	Wing Length	Culmen Length	Bill Depth	Head +Bill	Nostril
Coats I.	948±64.2[a] (40)	218±4.5[a] (37)	35.5±1.88[a] (19)	14.7±0.60 (19)	-	29.2±1.56 (19)
Akpatok I.	839±63.4[b] (30)	213±5.3[b] (30)	33.6±1.67[b] (30)	-	-	28.2±2.12 (30)
Kipako	929±78.5[a] (28)	217±6.2[a] (31)	34.9±1.92[a] (31)	14.1±0.58 (31)	103±3.3 (31)	-
F	23.6	7.3	7.1	11.6	-	3.8
P	0.0005	0.005	0.01	0.0065	-	ns
F_{ST}	0.331	0.134	0.155	0.195	-	0.075
W. Atlantic	909±82.7 (98)	217±5.7 (98)	34.6±1.96 (80)	14.3±0.64 (50)	103±3.3 (31)	28.6±1.90 (49)
Hornøya	991±58.7 (67)	221±5.5 (66)	37.6±1.79 (67)	14.5±0.72 (67)	105±3.0 (67)	-
F	49.1	29.8	96.6	2.1	12.9	-
P[1]	0.0005	0.0005	0.0005	ns	0.0025	-
F_{ST}	0.232	0.155	0.400	0.018	0.119	-

bills, and narrower bills than those from two neighboring colonies (Digges and Hantzsch Islands). Similarly, Storer (1952) reported a north-south cline in wing length, bill length and bill depth among western Atlantic colonies, and Birkhead and Nettleship (1981) reported significant differences in mass and bill dimensions between two neighbouring Canadian colonies.

PROTEIN ELECTROPHORESIS

Only three of 20 proteins were polymorphic (Table 2.6; Fig. 2.2): ALB, G6PD and 6PGD. ALD and G6PD could only be scored from samples from Hornøya in 1989 and were excluded from further analyses. Proportion of loci polymorphic (0.11) and expected average heterozygosity (0.030) were almost identical to values for four Pacific thick-billed murres (0.13 and 0.031 respectively, Watada *et al.* 1987), and were slightly lower than means for Atlantic puffins (*Fratercula arctica*) from five northeastern Atlantic colonies (0.16 and 0.043 respectively; Moen 1991; although these studies involved several different proteins). All these values are slightly lower than the means reported by Evans (1987; 0.24 and 0.065 respectively) for other avian species.

Genotype frequencies did not depart from Hardy-Weinberg equilibrium for 6PGD at any colony, or for ALB at either Coburg or Coats Islands (X^2, $P > 0.10$). A significant heterozygote deficiency for ALB at Hornøya ($X^2_5 = 14.9$, $P < 0.001$; fixation index, $F = 0.211$) appeared to relate to a complete absence of AC heterozygotes (Table 2.6). Heterozygote deficiencies may result from mixing of two or more populations ('Wahlund effect'), inbreeding, assortative mating, or selection. Inbreeding should produce heterozygote deficiencies for all loci, but G6PD and 6PGD were not out of Hardy-

TABLE 2.6: Genotype frequencies and genetic variability measures for thick-billed murres sampled from four Atlantic colonies. P = percent loci polymorphic; $\overline{H}_O$ = observed mean heterozygosity; $\overline{H}_E$ = expected mean heterozygosity assuming Hardy-Weinberg equilibrium.

	Geno-type	Coats I.	Akpatok I.	Coburg I.	Northwest Atlantic	Hornøya
ALB	*n*	34	29	20	83	81
	AA	11	11	11	33	43
	AB	15	17	8	40	21
	AC	0	0	0	0	0
	BB	3	0	1	4	2
	BC	5	1	0	4	2
	CC	0	0	0	0	4
6PGD	*n*	35	29	20	84	81
	AA	34	29	20	83	79
	AB	1	0	0	1	2
	BB	0	0	0	0	0
$\overline{X}$ alleles/locus		1.2	1.1	1.1	1.2	1.2
(SD)		(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
P		11.1	5.6	5.6	11.1	11.1
$\overline{H}_O$		0.034	0.034	0.022	0.031	0.023
(SD)		(0.033)	(0.034)	(0.022)	(0.031)	(0.022)
$\overline{H}_E$		0.033	0.026	0.021	0.028	0.029
(SD)		(0.031)	(0.026)	(0 021)	(0.027)	(0.028)

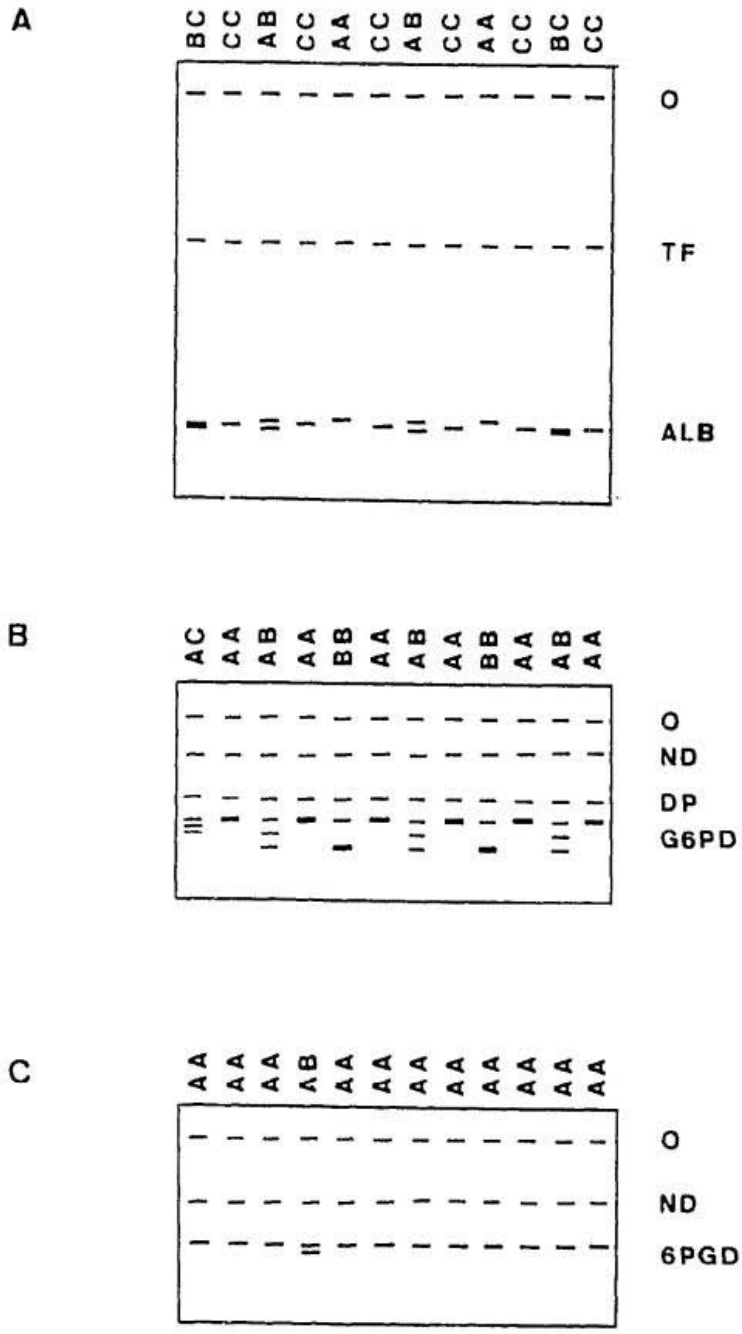


FIG. 2.2: Diagram of cellulose acetate gels stained for A) general proteins, B) G6PD and C) 6PGD. DP = degradation product; O = origin; ND = nonspecific dehydrogenase. (A degradation product with mobility similar to the A electromorph also occurred among inidividuals homozygous for the G6PD B electromorph.)

Weinberg equilibrium within Hornøya. A Wahlund effect is unlikely given the co-occurrence of birds with A or C alleles on individual ledges; birds with A *vs.* C alleles also did not differ in any of five morphological measurements (ANOVA, $P > 0.10$), or in cytochrome *b* genotype frequencies (X_2, $P > 0.10$). Disassortative mating for A and C alleles for albumin or a linked locus, and selection against AC heterozygotes also seem unlikely, but elimination of these possibilities would require more detailed analyses (e.g. typing of parents and offspring). The apparent absence of AC heterozygotes may be an electrophoretic artifact. Comparison of observed genotype frequencies with frequencies expected assuming Hardy-Weinberg equilibrium revealed an excess of BC heterozygotes. This suggests the possibility that some AC heterozygotes were scored as BC. Repeated electrophoresis of these samples did not resolve the discrepancy.

ALB allele frequencies differed slightly between Hornøya and the western Atlantic ($X_2^2 = 8.74$, $P < 0.05$; Table 2.6). This may have been an artifact of the difficulty in scoring AC and BC heterozygotes. An artificial data set was therefore created assuming that some AC heterozygotes were scored as BC so as to minimize the difference in allele frequencies among colonies; allele frequencies still differed significantly between Hornøya and the western Atlantic colonies ($X_2^2 = 8.47$, $P < 0.05$). However, Nei's (1978; 0.000) and Roger's (1972; 0.007) genetic distances, and Wright's (1978) $\overline{F}_{ST}$ (0.009) were all very low, indicating little genetic differentiation across the Atlantic. The three western Atlantic colonies also did not appear to differ genetically (Tables 2.6 and 2.7; $\overline{F}_{ST}$ = 0.023).

TABLE 2.7: Nei's unbiased (1978; above diagonal) and Roger's (1972; below diagonal) genetic distances among three western Atlantic thick-billed murre colonies.

	Coats	Akpatok	Coburg
Coats	-	0.000	0.001
Akpatok	0.007	-	0.000
Coburg	0.011	0.004	-

ANALYSIS OF THE CYTOCHROME *B* GENE

Description of Genotypes

The nucleotide and inferred amino acid sequences of the 307 bp segment of the cytochrome *b* gene for the most commonly encountered Atlantic thick-billed murre genotype are given in Fig. 2.3. Nucleotides 254 to 307 were not scored for all samples. A total of 18 genotypes, defined by 16 variable nucleotide sites, were found within the remaining 253 bp among 239 thick-billed murres (Table 2.8; Figs. 2.4, 2.5). One genotype (UL1; Figs. 2.3, 2.4, 2.5; Tables 2.8, 2.9) occurred in most murres analyzed. Fifteen of the less frequent genotypes differed from the major type by only one or two substitutions ($\bar{p}$ = 1.01±0.44; Table 2.8; Fig. 2.5). The two most divergent genotypes (UL10 and UL17) differed by 6 substitutions (p = 2.4%). Mean and maximum percent sequence divergences among thick-billed murre cytochrome *b* genotypes were within the range for other species (Table 2.10). All 16 substitutions were transitions (Table 2.8). Twelve (75%) occurred in the third base of a codon and were silent, and four (25%) entailed first positions. Two of the first position substitutions involved leucines and did not alter the amino acid sequence. The remaining two resulted in amino acid replacements: in type UL9 an isoleucine replaced a valine at amino acid #34, and in type UL13 a threonine replaced an alanine at amino acid #35.

The observed preponderance of transitions over transversions, and of third position changes over first or second position changes agrees with the expected substitutional pattern within species for a protein-coding mitochondrial gene (Brown *et al.* 1982, Wilson *et al.* 1985a, Li *et al.* 1985, Kocher *et al.* 1989, Edwards and Wilson 1990, Carr and

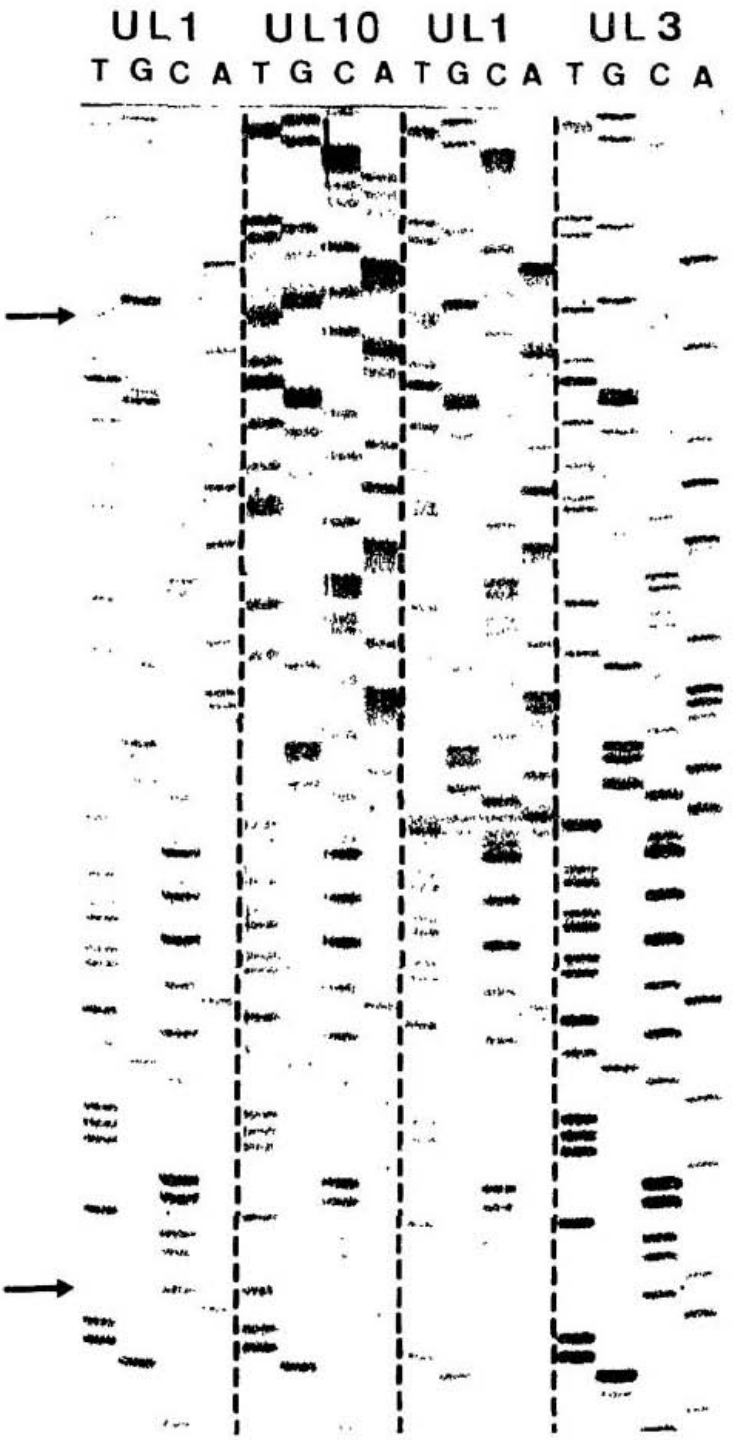
```
          phe gly ser leu leu gly ile cys leu leu thr gln ile leu  14
TBM    C TTC GGA TCC TTA CTA GGC ATC TGC CTA CTA ACA CAA ATC CTC  43
       | ||| ||| ||| ||| ||| ||  ||| ||| ||| ||| ||| ||| ||| |||
 CM    C TTC GGA TCC TTA CTA GGT ATC TGC CTA CTA ACA CAA ATC CTC  43
          phe gly ser leu leu gly ile cys leu leu thr gln ile leu  14

      thr gly leu leu leu ala thr his tyr thr ala asp thr thr leu  29
TBM ACA GGA CTC CTA TTA GCC ACA CAC TAC ACT GCA GAC ACA ACC CTA  88
      ||| ||| ||  ||   || ||| ||  ||| ||| ||| ||| ||| ||| ||| |||
 CM ACA GGA CTA CTC CTA GCC ACG CAC TAC ACT GCA GAC ACA ACC CTA  88
      thr gly leu leu leu ala thr his tyr thr ala asp thr thr leu  29

      ala phe ser ser val ala his thr cys arg asn val gln tyr gly  44
TBM GCC TTT TCA TCC GTT GCC CAC ACA TGT CGA AAC GTT CAA TAT GGC 133
      ||| ||| ||| ||| ||  ||| ||| ||| ||| ||| ||| ||  ||| ||| |||
 CM GCC TTT TCA TCC GTC GCC CAC ACA TGT CGA AAC GTA CAA TAT GGC 133
      ala phe ser ser val ala his thr cys arg asn val gln tyr gly  44

      trp leu ile arg asn leu his ala asn gly ala ser phe phe phe  59
TBM TGA CTG ATT CGA AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC TTC 178
      ||| ||  ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
 CM TGA CTA ATT CGA AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC TTC 178
      trp leu ile arg asn leu his ala asn gly ala ser phe phe phe  59

      ile cys ile tyr leu his ile gly arg gly phe tyr tyr gly ser  74
TBM ATC TGC ATT TAC CTC CAC ATT GGA CGA GGA TTC TAC TAC GGC TCG 223
      ||| ||| ||| ||| ||| ||| ||  ||| ||| ||| ||| ||| ||| ||| ||
 CM ATC TGC ATT TAC CTC CAC ATC GGA CGA GGA TTC TAC TAC GGC TCA 223
      ile cys ile tyr leu his ile gly arg gly phe tyr tyr gly ser  74

      tyr leu asn lys glu thr trp asn thr gly val ile leu leu leu  89
TBM TAC CTA AAC AAA GAA ACC TGA AAT ACA GGT GTC ATT CTC CTA CTA 268
      ||| ||  ||| ||| ||| ||| ||| ||  ||| ||| ||| ||  ||| ||| |||
 CM TAC CTG AAC AAA GAA ACC TGA AAC ACA GGT GTC ATC CTC CTA CTA 268
      tyr leu asn lys glu thr trp asn thr gly val ile leu leu leu  89

      ala leu met ala thr ala phe val gly tyr val leu pro        102
TBM GCC CTA ATA GCA ACC GCC TTC GTG GGT TAT GTT CTA CCA        307
      ||| ||| ||  ||| ||| ||  ||| ||  ||| ||  ||  ||  |||
 CM GCC CTA ATG GCA ACC GCT TTC GTA GGT TAG GTA CTC CCA        307
      ala leu met ala thr ala phe val gly tyr val leu pro        102
```

FIG. 2.3: Nucleotide and inferred amino acid sequences of a 307bp fragment of the cytochrome *b* gene for the most commonly encountered Atlantic genotypes of 235 thick-billed (TBM) and 130 common (CM) murres. Sites of nucleotide identity between the species are indicated by vertical bars. Nucleotides and amino acids in bold are sites of intraspecific variation.

TABLE 2.8. Eighteen cytochrome *b* genotypes found among thick-billed murres from five Atlantic and two Pacific colonies. Dots indicate identity with UL1. Nucleotide position from Fig. 2.3.

	Nucleotide Position																
Geno-type No.	22	31	61	86	91	100	101	104	112	115	124	133	137	196	202	208	223
UL1	C	A	C	C	C	C	G	G	A	T	T	C	C	C	A	A	G
UL2	"	"	T	"	"	"	"	"	"	"	"	"	"	"	"	"	"
UL3	"	"	"	"	"	"	"	"	"	"	C	"	"	"	"	"	"
UL4	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	G	"
UL5	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	A
UL6	"	"	"	"	"	"	"	"	"	"	"	"	"	"	G	"	"
UL7	"	"	"	T	"	"	"	"	"	"	"	"	"	"	"	"	"
UL8	"	"	"	"	"	"	"	"	"	C	"	"	"	"	"	"	"
UL9	T	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
UL10	"	"	T	"	"	"	"	"	"	"	"	"	"	T	"	"	"
UL11	"	"	"	"	"	T	"	"	"	"	"	"	"	"	"	"	"
UL12	"	"	"	"	T	"	"	"	"	"	"	"	"	"	"	"	"
UL13	"	"	"	"	"	"	"	A	"	"	C	"	"	"	"	"	"
UL14	"	"	"	"	"	"	A	"	"	"	"	"	"	"	"	"	"
UL15	"	"	"	"	"	"	"	"	G	"	"	"	"	"	"	"	"
UL16	"	"	"	"	"	"	"	"	"	"	C	"	T	"	"	G	"
UL17	"	G	"	"	"	"	"	"	"	"	C	"	T	"	"	G	"
UL18	"	"	"	"	"	"	"	"	"	"	"	T	"	"	"	"	"

FIG. 2.4: Autoradiogram of nucleotide sequences of part of the cytochrome *b* gene of four thick-billed murres. Arrows indicate variable sites.


UL1 UL10 UL1 UL3
T G C A T G C A T G C A T G C A

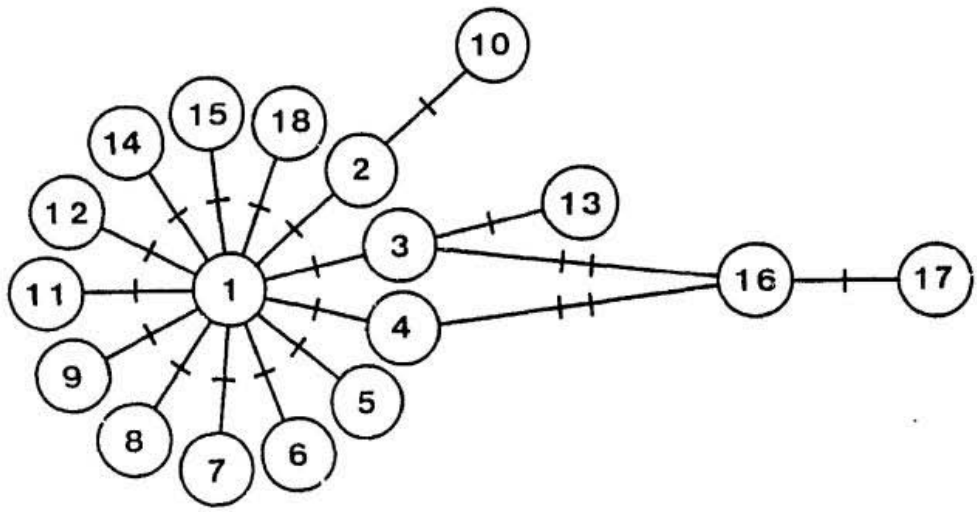


FIG. 2.5: Substitutional relationships among 18 cytochrome *b* genotypes from 239 thick-billed murres. Numbers of substitutions differentiating genotypes are indicated by numbers of cross-bars. Numbers within circles refer to genotypes listed in Table 2.8.

TABLE 2.9: Cytochrome *b* genotype frequencies, genetic diversities (H_0) and percent sequence divergence among individuals (π) for five Atlantic colonies and a Pacific sample of thick-billed murres.

Colony	Genotype																	Total	H_0	π
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
a) Total Numbers																					
Coats Island	43	1	16	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	65	0.51	0.23
Coburg Island	19	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	24	0.38	0.16
Akpatok Island	19	2	5	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	30	0.58	0.29
Kipako	19	0	9	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	31	0.55	0.24
Hornøya	59	6	9	0	1	5	1	0	1	0	1	0	0	0	0	0	0	2	85	0.50	0.23
Total Atlantic	159	10	41	1	2	11	1	1	1	1	1	1	1	1	1	0	0	2	235	0.51	0.23
Pacific	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	4	0.50	0.20
b) Percent Frequency																					
Coats Island	66	2	37	0	0	0	0	2	2	0	0	2	2	2	2	0	0	0			
Coburg Island	79	4	8	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0			
Akpatok Island	63	7	17	3	0	7	0	0	0	3	0	0	0	0	0	0	0	0			
Kipako	61	0	29	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0			
Hornøya	70	7	11	0	1	6	1	0	0	1	0	0	0	1	0	0	0	2			
Total Atlantic	68	4	17	0	1	5	0	0	0	0	0	0	0	0	0	0	0	1			
Pacific	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	25	0			

Table 2.10: Mean and maximum percent sequence divergence among cytochrome *b* genotypes within several vertebrates species, and genetic diversity indices.

Species	Number			*p*		H_0	Reference
	indiv-iduals	demes	geno-types	mean	max.		
Akodon aerosus	4	4	4	4.9	7.2	—	Smith and Patton 1991
A. subfuscus	3	3	3	5.2	7.2	—	Smith and Patton 1991
Branta canadensis	5	5	5	0.1	0.7	—	Quinn *et al.* 1991
Canis latrans	5	—	5	0.8	1.3	—	Wayne and Jenks 1991
C. lupus	3	3	3	0.4	0.5	—	Wayne and Jenks 1991
C. mesomelas	64	6	4	—	14.5	—	Wayne *et al.* 1990
C. rufus	7	6	5	2.5	5.0	—	Wayne and Jenks 1991
Gadus morhua	54	5	12	0.8	1.7	0.57	Carr and Marshall 1991
Odocoileus hemionus[1]	43	10	7	3.2	8.1	0.79	Carr and Hughes 1992
O. virginianus[2]	22	5	3	0.4	0.7	0.77	Carr and Marshall 1992
Pomatostomus temporalis	16	5	9	2.4	3.9	—	Edwards and Wilson 1990
Salmo salar	60	19	3	0.5	0.7	0.51	McVeigh *et al.* 1991
Thunnus alalunga	12	1	4	0.5	0.7	0.68	Bartlett and Davidson 1991
T. albacares	33	1	4	0.7	1.0	0.28	Bartlett and Davidson 1991
T. obesus	32	1	11	0.8	2.0	0.84	Bartlett and Davidson 1991
T. thynnus	33	1	6	0.6	1.0	0.28	Bartlett and Davidson 1991
Uria aalge	142	7	10	1.4	3.4	0.50	*present study*
U. lomvia	239	7	18	1.0	2.4	0.51	*present study*

1) Including type WTX.
2) Excluding type WTX.

Marshall 1991, Irwin *et al.* 1991). Both amino acid substitutions were conservative and/or occurred at sites not considered essential for enzymatic function (Dayhoff *et al.* 1979 *in* Weir 1990, Howell and Gilbert 1988, Kocher *et al*, 1989), and have been seen in the nucleotide sequences of the cytochrome *b* gene of other vertebrates (Kocher *et al.* 1989, Edwards and Wilson 1990, Irwin *et al.* 1991). The third position changes and probably all first position changes are therefore essentially neutral to selection.

Nucleotide sequences of 38 DNA samples did not differ between replicates. These results, as well as the observed substitutional pattern, underline the technique's reliability: if substitutions did not represent true genotypic variants but arose as amplification or sequencing errors, they would be randomly distributed with respect to both codon position and amino acid (Kocher *et al.* 1989).

Geographic Distribution of Genotypes

Atlantic and Pacific genotypes formed separate groups (Table 2.9; Fig 2.5): the most common Pacific variant (UL16) differed from the most common Atlantic variant (UL1) by 3 substitutions, or 1.8% ($\bar{p}$ = 1.67$\pm$0.38%; Table 2.8). Few thick-billed murres were sampled from the Pacific, but both the substitutional relationships among genotypes and genotype frequencies indicated significant differentiation between Atlantic and Pacific populations (X^2_{17} = 238, $P < 0.001$; G_{ST} = 0.30; γ = 1.00; π = 1.06%).

Wilson *et al.* (1985a) argued that mtDNA nucleotide sequences diverge with roughly clock-like regularity at a rate of about 2% per million years (see also Shields and Wilson 1987b). Assuming that sequences within the present 253 bp region of the cytochrome *b* gene evolve at about the same rate as the mean for the mitochondrial

genome (Quinn *et al.* 1991), $\bar{p}$ between Atlantic and Pacific thick-billed murre genotypes suggests that these genomes would have diverged ~0.8mya (range = 0.2—1.2mya).

Four genotypes (UL1, 2, 3, 6) occurred in at least four of five Atlantic colonies (Table 2.9). One of these (UL1) represented 68% of samples, and the other three involved 26%. The remaining 12 Atlantic genotypes occurred in only one or two individuals each, and constituted 6% of samples. Genetic diversity was lowest at Coburg Island (where sampling was most restricted), but otherwise was similar among colonies (Table 2.10). Although few population analyses of nucleotide sequence variation in the cytochrome *b* gene are available, genetic diversity was similar to other species (Table 2.10).

Neither the substitutional relationships among genotypes nor genotype frequencies provided any evidence for genetic differentiation either among western Atlantic colonies (G_{ST} = 0.003; γ = 0.007; π = —0.01) or between the eastern and western Atlantic (G_{ST} = 0.004; γ = 0.007; π = 0.00%), despite large sample sizes ($X^2 > 0.05$; Table 2.10). Percent sequence divergence among Atlantic genotypes was low ($\bar{p}$ = 0.84±0.24). Assuming a constant divergence rate of 2%/my (Shields and Wilson 1987b, Quinn *et al.* 1991), these genotypes would have diverged from a common ancestor ~0.4mya.

CONCLUSIONS

Cytochrome *b* genotypes suggest that Atlantic and Pacific populations of thick-billed murres are genetically differentiated (G_{ST} = 0.30; γ = 1.00; $\bar{p}$ = 1.67%; π = 1.06%), and that this differentiation occurred during the Pleistocene (~0.8mya). This agrees with both their subspecific classifications and their geographic separation. In contrast, analyses of proteins and nucleotide sequences of the cytochrome *b* gene indicated little genetic

differentiation among five colonies spanning the Atlantic, despite both morphological differentiation among colonies (Storer 1952, Birkhead and Nettleship 1981, Gaston *et al.* 1984; *present results*), and strong natal philopatry (Kampp 1988, Noble *et al.* 1991). The very weak genetic differentiation of Atlantic thick-billed murres also contrasts with structuring in other avian species with similar life histories and zoogeographies. Canada geese (Van Wagner and Baker 1986, 1990, Shields and Wilson 1987a), black brant (Shields 1990) and fairy prions (Ovenden *et al.* 1991) are all strongly philopatric and/or occur within areas that were glaciated within the past 10,000 years, and all exhibit genetic differences among local populations[1]. Data for thick-billed murres are more similar to results for red-winged blackbirds (Ball *et al* 1988, Gavin *et al.* 1991) and common grackles (*Quiscalus quiscula*, Zink *et al.* 1991a), which appear to be genetically homogeneous throughout North America despite substantial morphological differentiation.

The weak genetic differentiation in Atlantic thick-billed murres, despite strong natal philopatry and phenotypic differentiation among colonies, may result from gene flow and/or large founder populations coupled with ecophenotypic effects. Rates of genetic exchange as low as two females per generation may be sufficient to counteract genetic drift in mtDNA frequencies (Birky *et al.* 1983), and would be practically impossible to detect with banding studies in this species. Recent founding of colonies by large numbers of individuals may also result in genetically similar colonies, regardless of current

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1) These studies all involved RFLP analysis of mtDNA, which appears to reveal approximately as much variation as cytochrome *b* sequences analysis (compare Tables 2.10 [p. 37] *vs.* 6.3 [p. 105], 5.2 [p. 77] *vs.* 6.4 [p. 108]). Sample sizes were also larger in the present study.

levels of gene flow (Mayr 1970, Slatkin and Maddison 1989). Most modern colonies of thick-billed murres are probably less than 10,000 years old, and murres do not breed until 4 or 5 years (Hudson 1985, Noble *et al.* 1991), so colonies probably have not yet attained equilibrium between mutation and migration rates.

Phenotypic differences could exist among colonies, despite moderate levels of gene flow or large founder populations, if environmental differences exist among colonies. The present colonies differ slightly in such influences as climate, ice conditions and diet (e.g. World Ocean Atlas 1979, Bradstreet and Brown 1985, Gaston 1985b, Gaston *et al.* 1987), and this variation may produce phenotypic differences due to selection pressures, environment-genotype interactions, or non-heritable variation such as in chick growth (e.g. Birkhead and Nettleship 1981, Gaston *et al.* 1983, 1987, Harris and Birkhead 1985, Hunt *et al.* 1986). For example, mean masses, wing lengths and culmen lengths of thick-billed murres in the present study were inversely correlated with log colony size ($r^2 = 0.88$, $P = 0.06$; $r^2 = 0.94$, $P = 0.03$; and $r^2 = 0.96$, $P = 0.02$ respectively; Tables 2.1, 2.5), in agreement with prey depletion theory (Ashmole 1963, Hunt *et al.* 1986, Birt *et al.* 1987). However, the possibilities that phenotypic differences result from founder effects, drift or rapid evolution in genes controlling morphology cannot be ruled out at present. More definitive answers may be obtained from analysis of a locus either that was affected by founder events or that has a very high mutation rate (e.g. the mitochondrial displacement loop [e.g. Aquadro and Greenberg 1983, Vigilant *et al.* 1989], single-copy hypervariable sites [e.g. Gibbs *et al.* 1991] or repeat sequences within the nuclear genome [e.g. Burke and Bruford 1987]).

CHAPTER III

GENETIC STRUCTURE OF COMMON MURRES

INTRODUCTION

Common murres are very similar to thick-billed murres in most biological aspects (Tuck 1960, Nettleship and Birkhead 1985). Most importantly, population parameters with the greatest influence on population genetic structure are virtually identical (Chapter I). Nonetheless, the species differ in the extent of morphological differentiation across the Atlantic. Atlantic thick-billed murres are classified as monotypic, but sufficient morphological variation exists among colonies of common murres to result in taxonomic subdivision of this population into at least two subspecies: a small, brownish *albionis* occurring in the mid-eastern Atlantic, and a larger, darker *aalge* in the northeastern and western Atlantic (reviewed in Bédard 1985). Various authors (reviewed in Bédard 1985) also describe a very dark *hyperborea* from the Barents Sea, a type intermediate between *aalge* and *albionis* (*intermedia*) from the Baltic, a darker subset of *aalge* (*spiloptera*) from the Faeroe Islands, and a very light *ibericus* from the Iberian Peninsula. Thick-billed murres nest continuously throughout the Arctic, but Atlantic and Pacific common murres are geographically separated, and another subspecies of common murre, *inornata*, is recognized from the Pacific Ocean (Tuck 1960). These differences in phenotypic variation between thick-billed and common murres suggest that their population genetic structures may also differ.

The current chapter presents an investigation of genetic structure within the Holarctic population of common murres, using direct sequence analysis of part of the mitochondrial cytochrome *b* gene. Atlantic and Pacific subspecies of common murres were predicted to be genetically distinct, and genetic differentiation of common murres across the Atlantic was predicted to be greater than that of thick-billed murres.

METHODS

Common murres were sampled at two colonies in the northwestern Atlantic (Witless Bay, including Green and Gull Islands, and Funk Island, all in Newfoundland), and two in the northeastern Atlantic (Fair Isle in Scotland, and Hornøya in Norway; Table 3.1; Fig. 3.1). The first three colonies are within the range of *U. a. aalge*, whereas Hornøya occurs in the range of *U. a. hyperborea*. At all colonies, adults were noosed on breeding ledges, 1—5mL blood was taken from a brachial vein, and birds were banded and released. In addition, liver was obtained from four adults that were shot within ~1km of the breeding cliffs at Witless Bay, and from 10 chicks from Funk Island. Blood collected at Funk Island was added either to an equal volume of 10% EDTA or to 14mL of lysis buffer (Longmire *et al.* 1988; Chapter II), and was stored at ambient temperatures. All other samples were collected into Vacutainers^{R} and stored below —20°C.

Morphological measurements (Chapter II) were made on murres captured for blood sampling at Funk Island in 1990 and at Hornøya in 1998. Each researcher also measured 10 common murre skins from the Royal Ontario Museum (Toronto, Ontario) to corrected for measurer effects (Chapter II). Significant differences were found in bill depth

TABLE 3.1: Colony locations and sizes, and numbers of common murre samples.

Colony	Location	Colony Size (pairs)	Sample Size[1]	Measurer[2]
Funk Island	49°45'N, 53°11'W	77,500[3]	27 29	WAM PR
Witless Bay	47°14'N, 52°47'W	396,000[3]	22	
Fair Isle	59°32'N, 01°39'W	32,320[4]	24	
Hornøya	72°22'N, 31°10'E	1,900[5]	32	RTB
Big Koniuji	52°10'N,159°31'W	6,400[6]	7	
Semidi Islands	56°00'N,156°45'W	1,020,000[6]	2	
Agattu Island	52°20'N,174°25'E	10,750[6]	3	

1) Not all birds were both bled for DNA analyses and measured.
2) WAM = W.A. Montevecchi; PR = P. Ryan; RTB = R.T. Barrett.
3) Cairns *et al.* 1989.
4) Birds counted on cliffs; Heubeck *et al.* 1991.
5) R.T. Barrett (Univ. Tromsø, *pers. comm.*).
6) Birds counted on cliffs; Sowls *et al.* (1978).

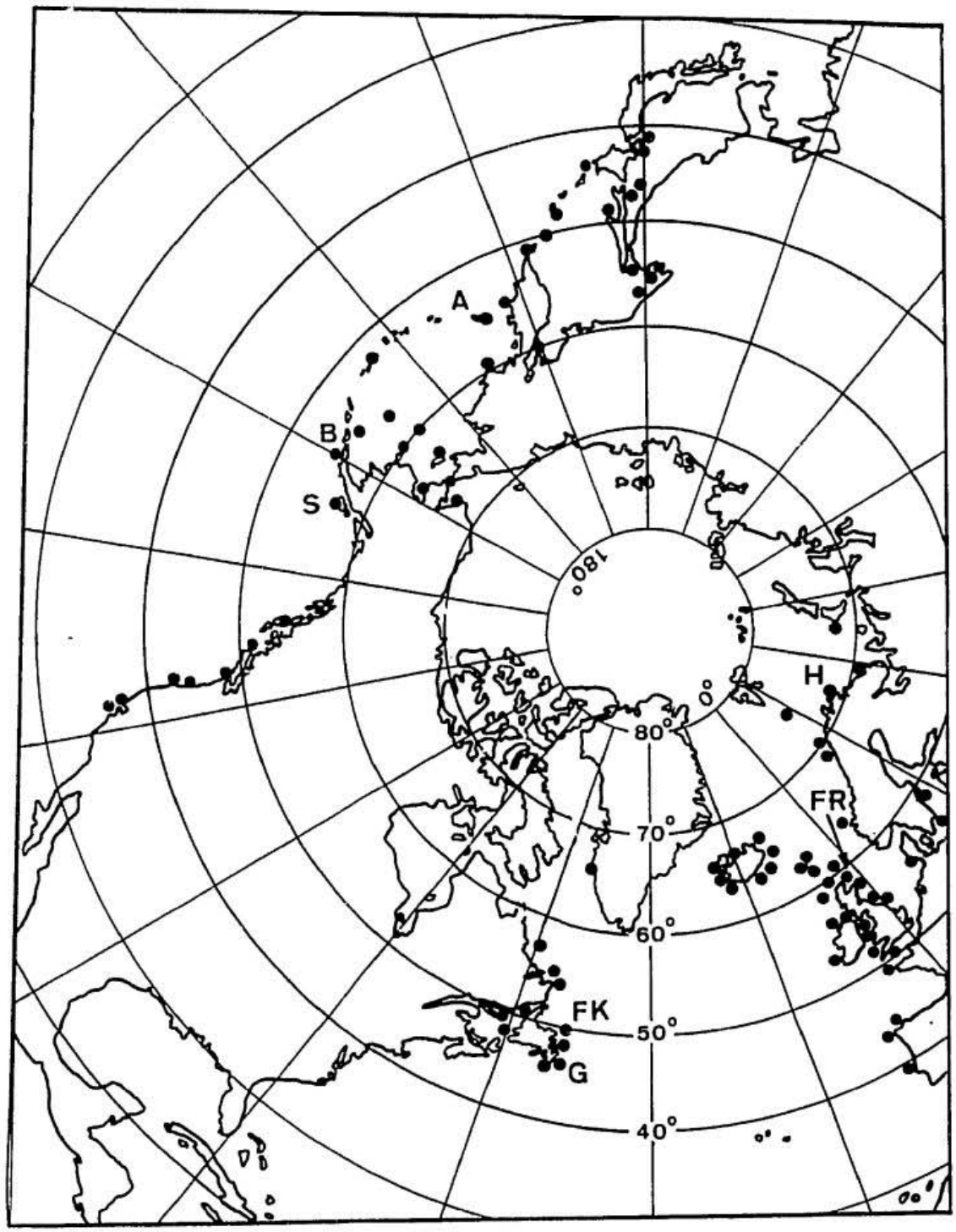

FIG. 3.1: Breeding colonies of common murres. Dots represent colonies. A = Agattu Island; B = Big Koniuji; FK = Funk Island; FR = Fair Isle; G = Great and Gull Islands; H = Hornøya; S = Semidi Islands. Redrawn from Tuck (1960).

measurements between P. Ryan and R.T. Barrett, and in head+bill measurements between W.A. Montevecchi and P. Ryan (Table 3.2). R.T. Barrett's measurements of culmen were also slightly but not significantly greater than those of either W.A. Montevecchi or P. Ryan. Measurements for live birds for these three variables were therefore corrected by decreasing or increasing the values for the researchers with largest and smallest mean skin measurements (respectively) by their mean difference from the intermediate mean. Presence of a bridle (a white eye ring and auricular groove) was also recorded.

Protocols for DNA extractions, amplifications, sequencing and statistical analyses were similar to those for analyses of thick-billed murres (Chapter II) except that the annealling step for double-stranded amplifications was conducted at 50°C.

RESULTS AND DISCUSSION

MORPHOMETRICS

Analysis of variance on adjusted morphological measurements revealed highly significant differences between murres from Funk Island and Hornøya (Table 3.3), with birds from Hornøya being larger in all dimensions. (Significant differences were also found for all comparisons using uncorrected measurements.) The mean among-colony component of morphological variation was also high ($\overline{F}_{ST}$ = 0.43±0.13, n = 5 measurements). A significantly larger proportion of murres were bridled at Hornøya (31% of 32 murres) than at Funk Island (14% of 59; X_1^2 = 4.09, $P < 0.05$). The marked morphometric differences between these colonies agrees both with reported plumage variation and with different subspecific classifications (Bédard 1985).

TABLE 3.2: Means (±SD) of four measurements made by three researchers on 10 common murre skins, and results of ANOVA (treatment x subjects, unbalanced design). Means with different letter superscripts differ according to Tukey's studentized range test.

Measurer[1]	Wing	Culmen	Depth	Head+Bill[2]
RTB	201±7.7	44.12±1.943[a]	12.95±0.542[b]	11.10±0.356
WAM	200±9.5	43.59±2.326	12.76±0.644[a]	11.26±0.695[a]
PR	203±8.2	43.05±3.334[b]	12.96±0.653[b]	10.80±0.705[b]
F	-	3.11	4.41	4.27
P	ns	0.069	0.028	0.04

1) WAM = W.A. Montevecchi; PR = P. Ryan; RTB = R.T. Barrett.
2) $n = 4$ skins.

TABLE 3.3: Measurement means (±SD), results of ANOVAs, and F_{ST}s for common murres at Funk Island and Hornøya. Mass is in g; all other measurements are in mm. Sample sizes are in parentheses.

	Measurement				
Colony	Mass	Wing	Culmen	Bill Depth	Head +Bill
Funk Island	890±51.7 (27)	209±4.8 (29)	43.3±2.41 (29)	13.2±0.55 (29)	111±3.1 (29)
Hornøya	1016±76.1 (31)	213±4.1 (32)	47.5±2.43 (31)	14.3±0.59 (32)	118±3.8 (32)
F	52.7	15.0	44.9	63.8	65.9
P	0.0005	0.0015	0 0005	0.0005	0.0005
F_{ST}	0.485	0.203	0.436	0.520	0.527

ANALYSIS OF THE CYTOCHROME *B* GENE

Description of Genotypes

Fig. 2.3 (p. 30) presents the nucleotide and inferred amino acid sequences of the most commonly encountered Atlantic common murre genotype. Nucleotides 205 to 307 were not scored for all common murres. Ten cytochrome *b* genotypes, described by 13 variable nucleotide sites, were found within the remaining 204bp among 142 common murres (Table 3.4; Fig. 3.2). Mean p (1.40±0.87%) was within the range of values for other species studied using similar laboratory protocols (Table 2.10, [p. 37]).

The 10 genotypes formed 2 distinct lineages: UA3 differed from UA1 by 5 substitutions (p = 2.5%), including three third position-, one second position- and one first position transition. The first and second position mutations produced amino acid substitutions: methionines (sulfur-containing side chain) replaced both a threonine (aliphatic hydroxyl side chain) at amino acid #21 and a valine (aliphatic side chain) at position #41. Neither type of substitution is unusual (Dayhoff *et al.* 1979 *in* Weir 1990). Seven of the eight remaining genotypes differed from UA1 by only one or two third position transitions (p = 0.49—0.98%); genotype UA10 differed from UA1 by a first position transition that replaced an isoleucine with a valine at position #77. The most divergent genotypes (UA3 *vs.* UA5 and UA9) differed by seven substitutions (p = 3.4%). Maximum p for common murres was within the range of values for other species studied using similar laboratory protocols (Table 2.10 [p. 37]). All substitutions are probably essentially neutral to selection (see Chapter II).

TABLE 3.4: Ten cytochrome *b* genotypes found among four Atlantic and three Pacific colonies of common murres. Dots indicate identity with UA1.

	Nucleotide Number												
Geno-type No.	1 9	2 0	4 0	6 1	6 3	6 4	7 0	7 9	1 2 1	1 2 2	1 4 5	1 6 0	2 0 2
UA1	T	A	C	C	C	G	C	C	C	G	A	C	A
UA2	"	"	"	"	"	A	"	"	"	"	"	"	"
UA3	"	"	"	"	T	"	T	T	"	A	"	T	"
UA4	"	"	"	T	"	"	"	"	"	"	"	"	"
UA5	"	"	"	T	"	"	"	"	"	"	G	"	"
UA6	"	"	T	"	"	"	"	"	"	"	"	"	"
UA7	"	"	"	"	"	"	"	"	"	"	"	"	G
UA8	"	"	"	"	"	"	"	"	T	"	"	"	"
UA9	C	"	"	T	"	"	"	"	"	"	"	"	"
UA10	"	G	"	"	"	"	"	"	"	"	"	"	"

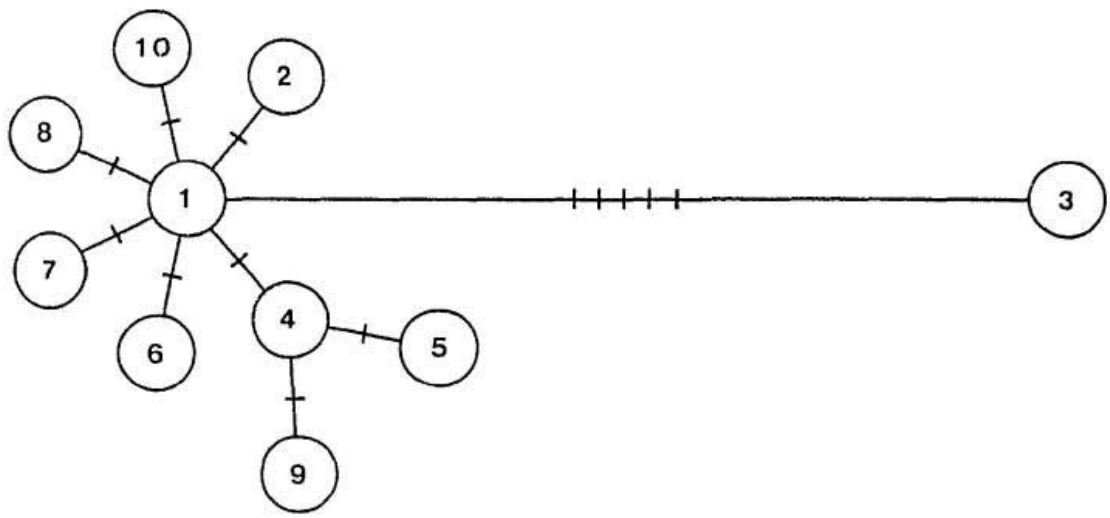

FIG. 3.2: Substitutional relationships among 10 cytochrome *b* genotypes of common murres. The number of substitutions distinguishing related types is depicted by cross-bars. Numbers within circles refer to genotypes listed in Table 3.4.

Differentiation of Atlantic vs. *Pacific Common Murres*

Ten (84%) of 12 Pacific common murres possessed one genotype, which also occurred in one Atlantic murre from Fair Isle (UA4; Table 3.5). Otherwise, no genotypes occurred in murres from both oceans. Although few common murres were sampled from the Pacific, both the substitutional relationships among genotypes and genotype frequencies indicated significant differentiation between Atlantic and Pacific populations ($X^2_9 = 130$, $P < 0.001$; $G_{ST} = 0.47$; $\gamma = 0.99$; $\bar{p} = 1.37 \pm 3.43$; $\pi = 0.64\%$; Tables 3.4, 3.5; Fig. 3.2). This genetic differentiation correlates with their morphological differences (reviewed in Tuck 1960, Bédard 1985). The phenotypic and genetic differentiation of birds from these oceans probably results from current and historical separation by Arctic water and ice, and the Bering Landbridge respectively (Flint 1971).

Assuming a clock-like rate of divergence of 2%/my (Wilson *et al.* 1985a, Shields and Wilson 1987b, Quinn *et al.* 1991), $\bar{p}$ between Atlantic and Pacific genotypes suggests these genomes have been separated for ~0.7my. This value is similar to the estimate for Atlantic and Pacific thick-billed murre genotypes (Chapter II).

Differentiation of Common Murres Within the Atlantic

Ninety-seven (75%) of 130 Atlantic common murres possessed genotype UA1 and 27 (21%) had type UA3; the other six Atlantic genotypes occurred in only one individual each, and comprised 5% of samples. These results are very similar to those of Moum *et al.* (1991), who examined mtDNA RFLPs in 51 common murres from four Norwegian colonies. They found that one composite genotype represented 70% of birds; 12 other genotypes differed from the major type by only one or two restriction site gains or losses,

TABLE 3.5: Cytochrome *b* genotype frequencies, genetic diversities (H_O) and percent sequence divergence among individuals (π) from four Atlantic colonies and a Pacific sample of common murres.

Colony	Genotype										Total	H_O	π
	1	2	3	4	5	6	7	8	9	10			
a) Total numbers.													
Witless Bay	12	1	9	0	0	0	0	0	0	0	22	0.56	1.29
Funk Island	40	0	13	0	0	0	0	1	0	1	55	0.42	0.94
Fair Isle	19	0	4	1	0	0	0	0	0	0	24	0.36	0.75
Hornøya	26	0	1	0	0	1	1	0	0	0	29	0.20	0.24
Atlantic	97	1	27	1	0	1	1	1	0	1	130	0.40	0.86
Pacific	0	0	0	10	1	0	0	0	1	0	12	0.33	0.06
Total	97	1	27	11	1	1	1	1	1	1	142	0.50	0.89
b) Percentage frequencies.													
Witless Bay	55	5	41	0	0	0	0	0	0	0			
Funk Island	73	0	24	0	0	0	0	2	0	2			
Fair Isle	79	0	17	4	0	0	0	0	0	0			
Hornøya	90	0	3	0	0	3	3	0	0	0			
Atlantic	75	1	21	1	0	1	1	1	0	1			
Pacific	0	0	0	83	8	0	0	0	8	0			
Total	68	1	19	8	1	1	1	1	1	1			

and occurred in only one bird each. There was no evidence for a secondary genotype equivalent to UA3 in Moum *et al.*'s (1991) data set; however, this genotype occurred in only 3% of Norwegian murres in the present study, so may have been absent from Moum *et al.*'s samples due to random sampling error.

Percent sequence divergence among Atlantic genotypes in the present study was low ($\bar{p}$ = 1.45±1.11 including UA3; 0.84±0.23 excluding UA3). Assuming a constant divergence rate of 2%/my (Wilson *et al.* 1985a, Shields and Wilson 1987b, Quinn *et al.* 1991), the Atlantic genotypes excluding UA3 diverged from a common ancestor ~0.4 mya, and all Atlantic genotypes diverged within ~0.7 my. The former number is identical to the estimate for Atlantic thick-billed murres.

Genetic diversity was similar among three of the Atlantic colonies, but was much lower within Hornøya than within either Funk Island or Witless Bay (Table 3.5). Diversities were higher than Moum *et al.*'s (1991) mtDNA RFLP estimates for four Norwegian colonies (range = 0.083—0.17), probably because of the different analytical techniques. Moum *et al.* (1991) hypothesized that low diversities in Norwegian common murres are due to repeated population bottlenecks, possibly because of dramatic fluctuations in prey populations. They found that diversity was especially low at Hornøya (H_O = 0.083), and attributed this to restricted gene flow and small population size at that colony. With the exception of Hornøya, genetic diversities in the present study were similar to those within colonies of thick-billed murres (range = 0.38—0.58; Table 2.10 [p. 37]). Genetic diversity within the overall Atlantic population of common murres (0.50) was almost identical to that for Atlantic thick-billed murres (0.51), and was within the

range for other species studied using cytochrome *b* sequence analysis (Table 2.10 [p. 37]).

Genotype frequencies were significantly different among the four Atlantic common murre colonies ($X^2_{12} = 30.1$, $P < 0.05$; $G_{ST} = 0.062$; $\gamma = 0.055$; Table 3.5), although percent sequence divergence was low ($\pi = 0.09$). Specifically, UA1 was more frequent at Hornøya (90% of 29 birds) than at Witless Bay (55% of 22 murres), and UA3 was more common at Witless Bay (41% of 22 birds) than at Hornøya (3% of 29 murres). Genotype frequencies appeared to vary along a southwest-northeast cline. These results are complemented by those of Moum *et al.* (1991), who found no differentiation in mtDNA RFLPs among four Norwegian common murre colonies.

The apparently clinal change in frequencies of genotypes UA1 and UA3 from Witless Bay northeast to Hornøya correlates both with morphological differentiation across the Atlantic (Tuck 1960, Bédard 1985, *present study*) and with a north-south cline in the incidence of bridling (Southern 1962, Birkhead 1984)[1]. Clines may result from many factors, such as founder effects, genetic bottlenecks or introgression following secondary contact. At least three possible scenarios may explain the clines in Atlantic common murres.

Selection.-Environmental factors may favour one phenotype at one end of a cline and another at the other end, with an intervening gradient resulting either from low levels of gene flow or from a selection gradient. Southern (1962) and Järvinen and Vepsäläinen (1973) correlated the bridling cline in common murres with sea surface isotherms, and

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1) There was no correlation between cytochrome *b* genotype and bridling among birds in the present study; X^2 test, $n = 84$, $P > 0.10$).

postulated that bridled murres have greater cold water tolerance. However, selection seems unlikely to explain the cline in cytochrome *b* genotypes. Firstly, the genotypic cline is not associated with any obvious oceanographic or climatic gradient (e.g. World Ocean Atlas 1979, Nettleship and Evans 1985). Secondly, most of the nucleotide substitutions differentiating UA1 and UA3 are silent, and both amino acid substitutions are probably essentially neutral to selection (Dayhoff *et al.* 1979 *in* Weir 1990, Kocher *et al.* 1989, Edwards and Wilson 1990, Irwin *et al.* 1991). The possibility exists that a linked mitochondrial gene differs between the two cytochrome *b* genotypes, and that the linked gene possesses a mutation with a selection coefficient that varies with latitude. This cannot be ruled out at present but seems unlikely given the generally conservative nature of mutations in mtDNA. Demonstration of maintenance of the genotypic cline by selection would require direct evidence that fitness differs between the two cytochrome *b* genotypes, and that this fitness differential varies with some environmental variable.

Founder Effects/Genetic Bottlenecks.-Clines may also result from low levels of gene flow in conjunction with either founder effects or genetic bottlenecks. Most modern common murre colonies occur in areas that were glaciated during the Wisconsin and Weischelian. During this time the birds probably nested in a more southerly refugial centre (consisting of one or more colonies). Founder effects may have resulted in progressively lower proportions of genotype UA3 as murres repopulated northward (see also Southern 1962, Birkhead 1984, Price and Boag 1985). Sage and Wolf (1986) proposed a similar hypothesis to account for low genic diversity within Dall's sheep (*Ovis dalli*). This possibility is supported by an apparently clinal decrease in genetic diversity within

colonies with latitude (Table 3.5).

Secondary Contact and Introgression.-Clines in cytochrome *b* genotype frequencies and bridling in common murres may also be explained by secondary contact between two differentiated populations. Common murres may have survived the glaciations in two or more main refugial centres: one consisting mostly of birds with genotype UA1 and its derivatives (and bridles) in a polynya in the Norwegian and/or Barents Seas, and one or more with a high proportion of genotype UA3 (and few bridles) in the central Atlantic (possibly one on each side of the Atlantic). As the glaciers retreated, murres gradually spread from these refugia throughout the north Atlantic, and low levels of gene flow established a cline. Modern wintering ranges of birds often correspond to historical breeding grounds (reviewed in Gauthreaux 1975), and wintering areas of modern common murres are consistent with a multi-refugium scenario. Common murres have many regional wintering areas, most of which are slightly south and farther offshore from breeding colonies. Common murres from northern Norway and the Barents Sea winter in that region (Brown 1985a). Glutz von Blotzheim and Bauer (1982) suggested the existence of a refugium in the vicinity of the Barents Sea to account for the occurrance of two distinct forms of Atlantic puffins (one high Arctic and one Atlantic).

Samples from more common murre colonies may be informative. For example, a high incidence of UA3 at the southernmost colonies in Iberia would support a north-south selectional gradient, whereas a low frequency of UA3 would be consistent with a trans-Atlantic interuption in gene flow.

CHAPTER IV

GENETIC SUBSTRUCTURING WITHIN A THICK-BILLED MURRE COLONY

INTRODUCTION

Banding studies of several avian species indicate that recruits often breed not only in their natal colonies but also close to their natal sites (e.g. Laysan albatrosses *Diomedea immutabilis*, Fisher 1971, 1976; herring gulls *Larus argentatus*, Chabrzyk and Coulson 1976; northern gannets *Sula bassanus*, Nelson 1978; Atlantic puffins, Harris 1983). Such philopatry could lead to genetic differentiation within colonies. Genetic substructuring has never been measured but is important for several reasons. From a theoretical viewpoint, it may promote altruism. It could also result in inbreeding, which may affect fitness. From a practical standpoint, substructuring would require careful sampling regimes for among-colony genetic comparisons.

Noble *et al.* (1991) found direct evidence for philopatry to natal cliff ledges in thick-billed murres. Phenotypic differentiation has also been found within both thick-billed and common murre colonies (Birkhead *et al.* 1980, Gaston and Nettleship 1981, Birkhead 1985, A.J. Gaston *unpubl. data*), which suggests that genetic substructuring may exist. In the present chapter, morphometrics, protein electrophoresis and direct sequence analysis of part of the mitochondrial cytochrome *b* gene were used to examine the relationships among thick-billed murres breeding at different sites within each of three Atlantic colonies.

METHODS

In July 1989, six breeding thick-billed murres were sampled from each of six sites within Hornøya, Norway (Table 2.1 [p. 11]; Fig. 2.1 [p. 13]). Two sites (2E and 2W) were at opposite ends of one ledge (within ~5m), and a third (2B) was on a ledge directly below the first. The other sites (1, 3 and 4) were distributed throughout the colony. Thus, potential structuring could be measured 1) within a ledge, 2) between ledges within one area, and 3) among areas. In 1990, 9—10 additional murres were sampled from each site except site 1.

Less rigorous sampling was conducted within Coats Island (eastern Canada) and Kipako (western Greenland; Table 2.1 [p. 11]; Fig. 2.1, [p. 13]). At Coats Island, birds were sampled from four sites in the western subcolony ('S', 'D', 'L' and 'T') and one from the eastern subcolony ~1.5km distant (site 'Z', including three ledges; Gaston *et al.* 1987). At Kipako, adults were sampled from four areas spanning the colony length, including upper and lower ledges in one area. Sample sizes ranged from 5 to 15.

At all three colonies, adults were noosed on breeding ledges, measured, bled, banded and released (Chapter II). Protein electrophoresis was conducted on blood from Coats Island and Hornøya, and part of the cytochrome *b* gene was amplified and sequenced from all samples (Chapter II).

RESULTS AND DISCUSSION

MORPHOMETRICS

Wing lengths differed significantly among thick-billed murres breeding on the upper and lower ledges in area 2 at Hornøya ($\overline{X}$ = 220±4.7mm, n = 32 *vs.* 224±3.1mm,

n = 10 respectively; $F_{1,41}$ = 9.57, Bonferonni corrected P = 0.018); otherwise, no morphological differences were found among murres breeding at opposite ends of ledge 2U, on upper and lower ledges in area 2, or among the four collection areas (ANOVA for unbalanced design, $P > 0.10$). The among-population components of morphological variation (Barrowclough 1991; Chapter II) were low for all comparisons ($\overline{F}_{ST}$ = 0.064±0.068 between ends of ledge 2U, 0.056±0.079 between ledges in area 2, and 0.060±0.037 among the four collection areas; n = 5 measurements in all cases).

At Coats Island, significant differences in mass occurred between the western ($\overline{X}$ = 922±59.3g, n = 22) and eastern subcolonies ($\overline{X}$ = 985±51.3g, N = 15; $F_{1,39}$ = 10.1, Bonferonni corrected P = 0.015). However, samples from the western subcolony were collected in July 1987, whereas most of those from the eastern subcolony were collected in August 1990; differences in mass therefore may have been due to inter-seasonal and/or inter-annual variation (e.g. Gaston and Nettleship 1981, Croll *et al.* 1991). Otherwise, no morphological differences were found among either the five collection areas or the two subcolonies. $\overline{F}_{ST}$ (0.13±0.12) was slightly higher than at Hornøya, probably because of the variation in mass. No morphometric differences were found among collection sites within Kipako, and $\overline{F}_{ST}$ was low (0.076±0.061).

NUCLEAR PROTEINS

Genotype frequencies for G6PD and 6PGD did not differ significantly from Hardy-Weinberg expectations at any site within Hornøya (Tables 4.1, 4.2). Albumin genotype frequencies were also in equilibrium within areas 1, 3 and 4. However, all sites within area 2 had a significant deficiency of heterozygotes, especially AC heterozygotes (Table

TABLE 4.1: Genotype frequencies for thick-billed murres from six sites within Hornøya.

Site		1	2					3	4
Ledge			U			B	Total		
End			E	W	Total				
ALB	*n*	6	15	16	31	13	44	15	16
	AA	2	10	12	22	8	30	5	6
	AB	2	3	2	5	2	7	6	6
	AC	0	0	0	0	0	0	0	0
	BB	0	0	0	0	1	1	0	1
	BC	2	1	1	2	2	4	2	3
	CC	0	1	1	2	0	2	2	0
G6PD	*n*	6	6	6	12	6	18	6	6
	AA	0	1	0	1	0	1	2	0
	AB	1	1	2	3	2	5	1	3
	AC	0	1	0	1	0	1	0	0
	BB	5	3	4	7	4	11	3	3
	BC	0	0	0	0	0	0	0	0
	CC								
6PGD	*n*	6	15	16	31	13	44	15	16
	AA	6	15	15	30	12	42	15	16
	AB	0	0	1	1	1	2	0	0
	BB	0	0	0	0	0	0	0	0

TABLE 4.2: Chi-square statistics and fixation indices (F) for deviations from Hardy-Weinberg equilibrium in albumin genotype frequencies at six sites within Hornøya.

Site	1	2					3	4
Ledge		U			B	Total		
End		E	W	Total				
Raw Data								
X_3^2	-	13.9	10.4	20.6	8.4	23.2	-	-
P	ns	0.003	0.014	0.000	0.04	0.000	ns	ns
Pooled Data[1]								
X_1^2	-	4.8	5.2	9.0	6.2	14.7	-	-
P	ns	0.029	0.023	0.003	0.013	0.000	ns	ns
F	-0.091	+0.330	+0.395	+0.361	+0.333	+0.359	+0.118	-0.011

1) Cells AB and AC pooled (heterozygotes involving the common allele), and cells BB, BC and CC pooled (heterozygotes and homozygotes involving rare alleles only).

TABLE 4.3: Wright's F statistics for thick-billed murres from six sites within Hornøya.

Comparison	$\overline{F}_{IS}$	$\overline{F}_{IT}$	$\overline{F}_{ST}$
Within Ledge	0.23	0.32	0.12
Between Ledges	0.20	0.21	0.02
Among Areas	0.05	0.09	0.04

4.2). This deficiency is probably an electrophoretic artifact, rather than evidence for inbreeding (see Chapter II).

Albumin allele frequencies differed slightly among the four collection sites within Hornøya ($X^2_3 = 10.3$, $P < 0.05$; Table 4.1), although this may result from incorrect scoring of AC and BC heterozygotes. However, if an artificial data set is created that 1) assumes that some AC heterozygotes were incorrectly scored as BC, and 2) minimizes the difference in allele frequencies among sites (see Chapter II), allele frequencies still differed significantly among sites ($X^2_2 = 8.04$, $P < 0.05$). Nonetheless, Wright's (1965, 1978) F statistics were low (Table 4.3), indicating little substructuring. Neither allele nor genotype frequencies provided any evidence for either inbreeding or genetic substructuring within the two subcolonies at Coats Island (Table 4.4; $\overline{F}_{IS} = -0.084$, $\overline{F}_{IT} = -0.055$, $\overline{F}_{ST} = 0.027$).

CYTOCHROME *B* GENOTYPE FREQUENCIES

Cytochrome *b* genotype frequencies at Hornøya did not differ between the east and west ends of ledge 2U — almost all birds possessed genotype UL1 (X^2 test, $P > 0.10$; $G_{ST} = 0.036$; $\gamma = 0.015$; Table 4.5). However, frequencies differed significantly between upper and lower ledges in area 2 ($X^2_7 = 14.3$, $P = 0.01$; $G_{ST} = 0.065$; $\gamma = 0.12$; Table 4.5). Specifically, while 29 (91%) of 32 murres on the upper ledge were UL1, only 8 (53%) of 15 murres on the lower ledge were UL1; only 1 (3%) murre on the upper ledge carried genotype UL6, whereas 3 (20%) of those on the bottom ledge had this type. Differences in genotype frequencies were more pronounced among the four collection areas ($X^2_{24} = 50.8$, $P = 0.002$; $G_{ST} = 0.15$; $\gamma = 0.28$). Specifically, 2 (12.5%) of 16 murres in area 4

TABLE 4.4: ALB and 6PGD genotype frequencies within two subcolonies at Coats Island.

Protein	Genotype	East	West	Protein	Genotype	East	West
ALB	*n*	22	12	6PGD	*n*	23	15
	AA	8	3		AA	22	15
	AB	11	4		AB	1	0
	AC	0	0		BB	0	0
	BB	2	1				
	BC	1	4				
	CC	0	0				

TABLE 4.5: Cytochrome *b* genotype frequencies among thick-billed murres from six sites within Hornøya.

Site	Genotype									Total
	1	2	3	5	6	7	11	15	19	
2UE	13	1	0	0	1	0	0	1	0	16
2UW	16	0	0	0	0	0	0	0	0	16
2U Total	29	1	0	0	1	0	0	1	0	32
2B	8	0	1	1	3	1	0	0	1	15
1	1	3	2	0	0	0	0	0	0	6
2 Total	37	1	1	1	4	1	0	1	1	47
3	9	0	6	0	0	0	1	0	0	16
4	12	2	0	0	1	0	0	0	1	16
Total	59	6	9	1	5	1	1	1	2	85

were UL2, whereas 0 of 47 in area 2 possessed this type; 6 (37.5%) of 16 birds in area 3 were UL3, whereas 0 of 16 birds in area 4 were this type, and only 1 (2%) of 47 in area 2 were this type; and, although sample sizes were small, 3 (50%) of 6 murres in area 2 had type UL2, whereas 0—12.5% of murres in other areas had this type. No genetic differentiation was found among collection sites within either Kipako (G_{ST} = 0.00; γ = 0.00; Table 4.6) or Coats Island (G_{ST} = 0.00; γ = 0.00; Table 4.7). However, differentiation would have to be extensive to be detected with the small sample sizes.

EVIDENCE FOR GENETIC SUBSTRUCTURING WITHIN MURRE COLONIES

Although morphometric analyses and protein electrophoresis provided little evidence for either genetic substructuring or inbreeding within Hornøya, cytochrome *b* genotype frequencies differed significantly among sites. These apparently contradictory results can be explained in at least three ways.

1) *Sampling Error*. Because mtDNA is effectively haploid, whereas nuclear DNA is diploid in birds, the number of mitochondrial genomes sampled was half the number of nuclear genomes. Apparent substructuring of cytochrome *b* genotypes may therefore be a Type I statistical error. This seems unlikely given the statistical probabilities of the genotype distributions.

2) *Effective Population Size*. Because of its haploid state and maternal inheritance, the effective population size of mtDNA is roughly 1/4 that of nuclear DNA. MtDNA is therefore affected by genetic bottlenecks, founder effects and restricted gene flow to a greater extent than is nuclear DNA (Birky *et al.* 1983, Wilson *et al.* 1985a). Population genetic differentiation is therefore more likely to exist in mtDNA than in nuclear genes

TABLE 4.6: Cytochrome *b* genotype frequencies among thick-billed murres from three sites within Kipako.

Site	Genotype			Total
	1	3	6	
SE	7	4	1	12
NE	4	2	2	8
N	4	1	0	5
Total	15	7	3	25

TABLE 4.7: Cytochrome *b* genotype frequencies among thick-billed murres from five sites within Coats Island.

Site	Genotype					Total
	1	3	8	14	15	
S	6	2	0	0	0	8
D	3	1	0	0	0	4
L	5	1	1	0	0	7
T	2	1	0	1	1	5
Total West	16	5	1	1	1	24
Z	10	5	0	0	0	15
Total	26	10	1	1	1	39

or their products. For example, Zink (1991) found genetic population differentiation in mitochondrial but not nuclear genes of fox sparrows *(Passerella iliaca)*.

3) *Sex-Biased Dispersal.* Whereas frequencies of nuclear alleles are homogenized by movements of either males or females, mtDNA is not affected by male dispersal. Male-biased gene flow can therefore result in mitochondrial differentiation and nuclear homogeneity. A classic example involves Canada geese, which pair on common wintering grounds but breed in the female's natal colony. Accordingly, fixed differences in mtDNA have been found among breeding populations of Canada geese (Shields and Wilson 1987a, Van Wagner and Baker 1990), but differentiation of nuclear genes across North America is weak (Van Wagner and Baker 1986).

Little is known about dispersal in murres, but in most bird species dispersal of females is at least as great as that of males (Greenwood 1980). Structuring of mtDNA genotypes within Hornøya therefore more probably results from the small effective population size of mtDNA.

Cytochrome *b* genotype frequencies at Hornøya agree with indirect evidence regarding genetic differentiation within murre colonies. Noble *et al.* (1991) reported that, of over 600 murres banded as chicks and subsequently recorded breeding at Coats Island, all returned to the study area where they were hatched. A.J. Gaston (*unpubl. data*) found significant morphometric differences among thick-billed murres breeding at different study areas within Digges Sound. Gaston and Nettleship (1981) reported that color morphs of thick-billed murre chicks ('summer' *vs.* 'winter') were nonrandomly distributed within Prince Leopold Island. Bridled common murres appear to have clumped distributions

within colonies (Birkhead *et al.* 1980). Furthermore, the background color and spotting pattern of eggs, which appear to be genetically determined, are nonrandomly distributed in colonies of both species (Gaston and Nettleship 1981; Birkhead 1985). Together, these observations indicate that murres often return to breed in natal areas within colonies, and that this philopatry can result in genetic substructuring. Ferris *et al.* (1983) and Plante *et al.* (1989a) reported similar patterns of microgeographic structuring in house mice (*Mus domesticus*) and meadow voles (*Microtus pennsylvanicus*), respectively.

EVOLUTION OF PHILOPATRY

Shields (1982) proposed three general models to explain the evolution of philopatry: according to *ecogenetic* models, philopatry increases fitness due to local adaptations within heterogeneous environments; *ecological* explanations emphasize somatic (direct, personal) costs and benefits of dispersal (e.g. increased predation *vs.* increased food sources); and according to *genetic* explanations, philopatry results from selection against disruption of the genetic environment (e.g. coadapted gene complexes) with outbreeding. Ecological explanations of philopatry are not satisfactory for thick-billed murres since somatic costs of breeding dispersal are probably negligible compared to costs of seasonal migrations. Ecogenetic and genetic reasons also seem unlikely given the apparent lack of genetic differentiation among colonies. Intense natal philopatry in murres may instead result from information factors: the best cue for a recruit regarding an ideal breeding location is its parents' success at its natal site (Shields 1983). Once established, natal philopatry may both promote and be maintained by kin selection.

INBREEDING

Philopatry to natal ledges in murres must ultimately lead to inbreeding. The theoretical and empirical evidence regarding the fitness effects of inbreeding in natural populations is ambiguous. Theoretically, matings between kin may result in expression of deleterious alleles, loss of heterozygous advantage, and/or production of genetically homogeneous offspring (reviewed in Shields 1982, Bateson 1983, Allendorf and Leary 1986, Hepper 1986, Ralls *et al.* 1986). Evidence exists for all these effects in animals, and inbreeding depression has been demonstrated in several wild populations (e.g. olive baboons *Papio anubis*, Packer 1979). However, inbreeding may also provide benefits by preventing disruption of local and intrinsic (genomic) adaptations, circumventing somatic costs of outbreeding (e.g. dispersal), ensuring behavioural compatability of mates, and increasing the inclusive fitness benefits of altruism (Coulson 1972, Shields 1982, Bateson 1983, Allendorf and Leary 1986, Hepper 1986, Ralls *et al.* 1986). Many studies of wild populations have failed to find evidence of inbreeding depression (e.g. fairy wrens *Malurus splendens*, Rowley *et al.* 1986; Chacma baboons *Papio cynocephalus*, Bulger and Hamilton 1988), and van Noordwijk and Schlaroo (1981) found that recruitment from incestuous pairs of great tits was actually higher than from outbred pairs. Moreover, both theoretical and empirical evidence suggest that successive generations of inbreeding can reduce the deleterious effects of inbreeding (Shields 1983; e.g. Mauritius kestrel *Falco punctatus*, Temple 1986; pukeko *Porphyrio melanotus*, Jamieson and Craig 1989), although contradictory evidence also exists (e.g. Sittman *et al.* 1966, Charlesworth and Charlesworth 1987). Intensive banding studies would help to determine the fitness effects

of inbreeding in murres.

ALTRUISM AND KIN SELECTION

Altruism is broadly defined as 'any behaviour that benefits the direct fitness of others rather than self' (Rushton *et al.* 1984). Much debate exists over the causes of altruism, but it is generally explained in terms of either kin selection (Hamilton 1964) or reciprocation (Trivers 1971). Murres frequently preen their neighbours, and Birkhead (1985) found that the number of preening bouts that individual murres gave each neighbour was directly correlated with the number of bouts received from that neighbour. Allopreening may therefore be explained in terms of reciprocation. Murres of both species also frequently brood neighbouring chicks, protect them from predators and occasionally feed them (Tuck 1960). Although allopreening, and protection and brooding of foreign chicks may have small fitness costs, murres feed their chicks only 2—5 times daily and may travel 50—100 km or more to obtain each meal (Gaston and Nettleship 1981, Gaston 1985a, Gaston *et al.* 1985, Cairns *et al.* 1987). Misdirected feeding would therefore be costly. Brooding, protection and feeding of foreign chicks would also have significant fitness benefits for the recipient parents. However, if genetic correlations exist within sites, these altruistic behaviours may be interpreted in terms of kin selection. If individuals return to natal ledges to breed, then ledges will consist more or less of family groups; allopreening, and brooding, protection and feeding of neighbouring chicks will therefore increase inclusive fitness. Increased inclusive fitness may in turn contribute to the maintenance of philopatry to natal ledges.

CONCLUSIONS

Although the present mtDNA results are indicative of genetic differentiation within Hornøya, definitive demonstration of genetic substructuring will require DNA fingerprinting (e.g. Aquadro and Greenberg 1983, Burke and Bruford 1987, Vigilant *et al.* 1989, Gibbs *et al.* 1991). More extensive genetic analyses, coupled with detailed records of altruistic behaviour, would provide insight into the importance of kin selection in the evolution of both altruism and philopatry. Determination of inbreeding and its costs and benefits will require intensive banding studies.

CHAPTER V

PHYLOGENETIC RELATIONSHIPS AMONG THE ATLANTIC ALCIDAE

INTRODUCTION

The auks comprise a distinct assemblage of Northern Hemisphere, oceanic, pursuit-diving birds. Despite the affinities among the auks, debate has surrounded their position within the class Aves. At various times over the past 100 years, they have been grouped with penguins, loons, grebes, or diving petrels, or placed in their own order (Table 5.1; reviewed in Sibley and Ahlquist 1990). They are generally considered a monophyletic assemblage, and are currently classified as a family (Alcidae) within the order Charadriiformes.

Confusion also exists over relationships among the auks (Table 5.1). The oldest definitive auk fossils date to the Middle or Upper Miocene, some 5—7 million years ago (mya), when several genera (*Pinguinus*, *Uria*, *Alle*, *Fratercula* and *Lunda*) were present in forms virtually identical to their Recent descendents (Bédard 1985). However, Bédard (1985) argued from paleogeographical evidence that the initial alcid radiation occurred during the relatively cool Mid- to Late Oligocene Era, some 25 mya. With the possible exception of *Uria* (Storer 1952), auks are thought to have evolved in the Pacific Ocean and then colonized the Atlantic. Bédard (1985) proposed that *Uria* differentiated into a cold-water *lomvia* in the Arctic and North Atlantic, and a more temperate *aalge* in the Pacific during the Upper Miocene (about 5—7 mya), with subsequent oceanic reinvasions

TABLE 5.1: Classification of taxa mentioned in Chapter V. (Source: American Ornithologists' Union 1983.)

Order	Family	Tribe	Genus	Species	Common Name
Gaviiformes	Gaviidae				Loons
Podicipediformes	Podicipedidae				Grebes
Procellariiformes	Pelecanoididae				Diving petrels
Sphenisciformes	Spheniscidae				Penguins
Charadriiformes	Charadriidae		*Pluvialis*	*squatarola*	Black-bellied plover
	Scolopacidae		*Calidris*	*alpina*	Dunlin
	Laridae		*Larus*	*argentatus*	Herring gull
			Rissa	*tridactyla*	Black-legged kittiwake
	Alcidae	Alcini	*Alca*	*torda*	Razorbill
			Uria	*aalge*	Common murre
			U.	*lomvia*	Thick-billed murre
		Allini	*Alle*	*alle*	Dovekie
		Cepphini	*Cepphus*	*grylle*	Black guillemot
			C.	*columba*	Pigeon guillemot
			C.	*carbo*	Spectacled guillemot
		Brachyramphini	*Brachyramphus*	*marmoratus*	Marbled murrelet
			B.	*brevirostris*	Kittlitz's murrelet
		Synthliboramphini	*Synthliboramphus*	*hypoleucus*	Xantus' murrelet
			S.	*craveri*	Craveri's murrelet
			S.	*antiquus*	Ancient murrelet
			S.	*wumizusume*	Japanese murrelet

Table 5.1, Cont'd.

Order	Family	Tribe	Genus	Species	Common Name
		Aethiini	*Ptychoramphus*	*aleuticus*	Cassin's auklet
			Cyclorrhynchus	*psittacula*	Parakeet auklet
			Aethia	*pusilla*	Least auklet
			A.	*pygmaea*	Whiskered auklet
			A.	*cristatella*	Crested auklet
		Fraterculini	*Cerorhinca*	*monocerata*	Rhinoceros auklet
			Fratercula	*cirrhata*	Tufted puffin
			F.	*arctica*	Atlantic puffin
			F.	*corniculata*	Horned puffin
Columbiformes	Columbidae		*Columba*	*livia*	Rock dove

during the Upper Pliocene and/or Pleistocene. Relationships among the auklets and murrelets are especially uncertain, and the relative affinity of the dovekie (*Alle alle*) to the murres *vs.* the auklets and murrelets is also debatable (Table 5.1; reviewed in Strauch 1985, Sibley and Ahlquist 1990).

Phylogenetic analyses of the alcids to date have been based mainly on morphology and natural history; few molecular data are available (reviewed in Sibley and Ahlquist 1990). In the present chapter, nucleotide sequences of part of the cytochrome *b* gene were compared among the six extant Atlantic alcids, four non-alcid Charadriiform species and a dove. Specifically, I examined 1) the pattern of nucleotide substitutions among the 11 species, and 2) the positions of the murres within the family Alcidae.

METHODS

Muscle or blood was collected from one specimen each of razorbill (*Alca torda*), dovekie, black guillemot (*Cepphus grylle*), Atlantic puffin, herring gull, black-legged kittiwake (*Rissa tridactyla*), black-bellied plover (*Pluvialis squatarola*), dunlin (*Calidris alpina*) and rock dove (*Columba livia*; all collected in Newfoundland; Table 5.1). Nucleotide sequences were obtained as described (Chapters II and III) except that both the heavy and light strands were sequenced, and the annealling step of the double-stranded amplification was conducted at 45°C for the razorbill. Phylogenetically informative sites were used to obtain most parsimonious trees using PAUP (Swofford 1990).

RESULTS AND DISCUSSION

EVOLUTION OF NUCLEOTIDE AND AMINO ACID SEQUENCES OF THE CYTOCHROME *B* GENE

Within the Genus Uria

The nucleotide sequences of the most commonly encountered Atlantic variants of thick-billed and common murres differed by 19 substitutions in 307bp (Fig. 2.3 [p. 31]; p = 6.2%). Most (n = 12) substitutions involved third position transitions, and 6 comprised third position transversions. Only 1 involved a first position, and none resulted in amino acid substitutions. Two nucleotide sites were variable within both species; i.e. parallel mutations occurred at sites 61 and 202 (Tables 2.8 [p. 32], 3.4; Fig. 2.3 [p. 31]). Substitutions at all other sites increased the inter-specific divergence ($\tilde{p}$ = 5.9±1.22%, range = 3.9—8.6%). Percent nucleotide divergence between the murre species is within the range for other congeneric species studied using the same protocol (Table 5.2).

Assuming that sequences within the present region of the cytochrome *b* gene diverge at a clock-like rate of about 2%/my (Wilson *et al.* 1985a, Shields and Wilson 1987b, Quinn *et al.* 1991), $\tilde{p}$ among nucleotide sequences of the cytochrome *b* gene suggests that the murre species diverged 2—4.3 mya ($\overline{X}$ = 3 mya). Divergence dates may also be estimated using data from protein electrophoresis: Gutiérrez *et al.* (1983) and Marten and Johnson (1986) argued that avian proteins diverge at a rate of 0.038 - 0.051 *D*/my, where *D* is Nei's (1978) distance[1]. Electrophoretic analysis of thick-billed and

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1) These numbers are based on one fossil only.

TABLE 5.2: Percent divergence among nucleotide sequences of the cytochrome *b* gene of congeneric species.

Genus	Number Species	$\bar{p}$	Reference
Amazona	4	8.1—10.4	Birt *et al.* 1992
Astatotilapia	3	0.0	Meyer *et al.* 1990
Branta	2	6.7	Quinn *et al.* 1991
Canis	3	11.4—16.3	Wayne *et al.* 1990
Canis	5	5.3	Wayne and Jenks 1991
Gadus	2	4.6	Carr and Marshall 1992
Larus	3	0.0	V.L.F. *unpubl. data*
Odocoileus[1]	2	3.2	Carr and Hughes 1992
Pomatostomus	5	6.2—12.1	Edwards and Wilson 1990
Prognathochromis	3	0.0	Meyer *et al.* 1990
Salmo	2	5.1	McVeigh *et al.* 1991
Stenella	2	5.7	Irwin *et al.* 1991
Thunnus	4	2.4—4.0	Bartlett and Davidson 1991
Uria	2	5.9	*present study*

1) Type WTX classified as *O. hemionus*.

common murres from Hornøya, Norway (Chapter II, V.L.F. *unpubl. data*) indicate a Nei's distance of 0.126. This suggests a species divergence date of 2.5—3.3 mya, which is in close agreement with the estimate obtained using nucleotide sequence of the cytochrome *b* gene. Watada *et al.* (1987) calculated a Nei's distance of 0.099 between four thick-billed and one common murre from the Pacific; this yields a species divergence date of 2.0—2.6 mya. All three estimates are slightly lower than Bédard's (1985) hypothesis of ~5 mya.

Within the Charadriiformes

The four nucleotides were roughly equally represented within the first codon position among the 10 Charadriiform species and dove (Table 5.3; Fig. 5.1). A slight excess of thymines occurred within the second position, possibly because of the high representation of leucines within this segment of the cytochrome *b* gene. (Leucine is encoded as CTN or TTR, and 17% of amino acids in this section comprised leucines in the species examined; Table 5.4; Fig. 5.2). A marked imbalance in base usage occurred within the third codon position: adenines and cytosines were heavily used (representing 36% and 47% respectively of 78 such sites within the 11 species), whereas guanines were seldom used (3% of third positions). Similar patterns were found in the same region of the cytochrome *b* gene among babblers (Edwards and Wilson 1990), passerines (Edwards *et al.* 1991), parrots (order Psittaciformes; Birt *et al.* 1992) and mammals (Irwin *et al.* 1991).

The imbalance in base usage was accompanied by an inequality in codon usage fo most amino acids (Table 5.4). At two-fold degenerate sites involving purines, codons

TABLE 5.3: Numbers of adenines, cytosines, guanines and thymines at first, second and third codon positions within a segment of the cytochrome *b* gene for 10 Charadriiform species and a rock dove.

Codon Position	1				2				3			
Base	A	C	G	T	A	C	G	T	A	C	G	T
Species												
Common Murre	18	21	18	20	16	18	16	27	29	39	2	8
Thick-billed Murre	18	20	18	21	16	18	16	27	29	37	2	10
Razorbill	18	21	18	20	16	18	16	27	30	32	2	14
Dovekie	18	22	18	19	16	18	16	27	28	37	2	11
Black Guillemot	18	21	18	20	16	18	16	27	31	37	0	10
Atlantic Puffin	19	19	18	21	16	17	16	28	28	38	2	10
Herring Gull	17	22	18	20	16	17	16	28	31	34	2	11
Black-legged Kittiwake	17	21	18	21	16	17	16	28	29	35	3	11
Dunlin	16	21	18	22	16	17	16	28	25	40	2	11
Black-bellied Plover	18	19	18	22	16	17	16	28	26	39	3	10
Rock Dove	16	21	19	21	16	18	16	27	25	38	4	11
$\overline{X}$	18	21	18	20	16	18	16	27	28	37	2	11
S.D.	.93	1.0	.30	.92	.00	.52	.00	.52	2.1	2.4	.98	1.4
%	23	27	23	27	21	23	21	35	36	47	3	14

```
Common Murre        C TTC GGA TCC TTA CTA GGT ATC TGC CTA CTA  31
Thick-bill          C TTC GGA TCC TTA CTA GGC ATC TGC CTA CTA  31
Razorbill           C TTC GGA TCC CTA CTA GGC ATC TGT TTA CTA  31
Dovekie             C TTC GGA TCC TTC CTA GGT ATT TGC CTG CTA  31
Guillemot           C TTT GGA TCC CTA CTA GGC ATT TGC CTA CTA  31
Puffin              C TTC GGA TCC CTA CTG GGT ATT TGC CTA ACA  31
Gull                C TTC GGA TCC CTA CTA GGC ATT TGC CTA CTA  31
Kittiwake           C TTC GGA TCC CTA CTA GCC ATC TGT CTA CTG  31
Dunlin              T TTC GGA TCA CTC CTA GGC ATC TGC CTC ATA  31
Plover              C TTT GGC TCT CTG CTA GGC ATC TGC CTA ATC  31
Dove                C TTT GGG TCC CTA CTA GGC ATT TGC TTG CTA  31

Common Murre  ACA CAA ATC CTC ACA GGA CTA CTC CTA GCC ACG CAC  67
Thick-bill    ACA CAA ATC CTC ACA GGA CTC CTA TTA GCC ACA CAC  67
Razorbill     ACA CAA ATC CTT ACA GGA CTC CTA CTA GCC ACA CAT  67
Dovekie       ACA CAA ATC CTT ACA GGA CTC CTA CTA GCC ACA CAT  67
Guillemot     ACA CAA ATC CTC ACA GGA CTT CTA CTA GCC ACA CAT  67
Puffin        ACA CAA ATC CTC ACA GGA CTC TTA CTA GCC ATG CAC  67
Gull          ACA CAA ATC CTA ACA GGA CTC CTG CTA GCT ATA CAT  67
Kittiwake     ACG CAA ATC CTA ACA GGA CTT CTG CTA GCC ATA CAC  67
Dunlin        ACA CAG ATC CTA ACT GGC CTC CTA CTT GCC ATG CAC  67
Plover        ACA CAA ATC CTA ACA GGA TTA CTA CTA GCC ATA CAC  67
Dove          ACT CAA ATC CTA ACC GGC TTA CTA CTC GCC GCA CAT  67

Common Murre  TAC ACT GCA GAC ACA ACA CTA GCC TTT TCA TCC GTC 103
Thick-bill    TAC ACT GCA GAC ACA ACA CTA GCC TTT TCA TCC GTT 103
Razorbill     TAC ACT GCA GAT ACA ACA CTA GCT TTC TCA TCC GTT 103
Dovekie       TAC ACT GCA GAC ACA ACA CTA GCC TTC TCG TCC GTC 103
Guillemot     TAC ACC GCA GAC ACA ACC CTA GCC TTC TCA TCC GTT 103
Puffin        TAC ACC GCA GAT ACA ACC CTA GCC TTC TCA TCC GTT 103
Gull          TAC ACC GCA GAC ACA ACC CTA GCC TTC TCA TCC GTC 103
Kittiwake     TAT ACC GCA GAT ACA ACC CTA GCT TTC TCA TTC GTC 103
Dunlin        TAT ACT GCA GAC ACA ACC CTA GCC TTC TCA TCC GTC 103
Plover        TAC ACT GCA GAC ACA ACC CTC GCC TTT TCC TCC GTC 103
Dove          TAC ACT GCA GAC ACC ACC CTA GCC TTT TCA TCC GTT 103

Common Murre  GCC CAC ACA TGT CGA AAC GTA CAA TAT GGC TGA CTA 139
Thick-bill    GCC CAC ACA TGT CGA AAC GTT CAA TAT GGC TGA CTG 139
Razorbill     GCC CAC ACA TGT CGA AAC GTA CAA TAT GGT TGA CTA 139
Dovekie       GCC CAT ACA TGT CGA AAC GTT CAA TAC GGC TGA CTA 139
Guillemot     GCC CAC ACC TGC CGA AAT GTT CAA TAT GGC TGA CTA 139
Puffin        GCC CAC ACA TGC CGA AAC GTC CAA TAT GGC TGA TTA 139
Gull          GCC CAC ACA TGT CGA AAC GTA CAA TAT GGC TGA CTA 139
Kittiwake     GCC CAC ACA TGT CGA AAT GTA CAA TAC GGC TGA CTA 139
Dunlin        GCC CAC ACA TGC CGA AAC GTA CAA TAC GGC TGA CTA 139
Plover        GCC CAT ACA TGT CGA AAC GTA CAG TAC GGG TGA TTA 139
Dove          GCA CAC ACA TGC CGA AAC GTA CAG TAC GGC TCG CTA 139
```

FIG. 5.1: Nucleotide sequences of a fragment of the cytochrome *b* gene for 10 Charadriiform species and a rock dove. Nucleotide differences from the sequence of *Uria aalge* are highlighted.

```
Common Murre  ATT CGA AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC 175
Thick-bill    ATT CGA AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC 175
Razorbill     ATT CGG AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC 175
Dovekie       ATT CGA AAC CTC CAT GCA AAC GGA GCA TCC TTC TTC 175
Guillemot     ATC CGA AAC CTC CAT GCA AAC GGA GCA TCA TTC TTC 175
Puffin        ATC CGA AAC CTT CAC GCA AAC GGA GCA TCA TTT TTC 175
Gull          ATC CGA AAC CTC CAC GCA AAC GGA GCG TCA TTC TTC 175
Kittiwake     ATC CGA AAC CTC CAC GCA AAC GGA GCA TCA TTC TTC 175
Dunlin        CTC CGC AAC CTA CAT GCA AAC GGA GCC TCA TTC TTT 175
Plover        CTT CGC AAC TTA CAT GCA AAC GGC GCA TCA TTC TTC 175
Dove          ATC CGA AAC CTC CAT GCA AAC GGA GCC TCA TTT TTC 175

Common Murre  TTC ATC TGC ATT TAC CTC CAC ATC GGA CGA GGA TTC 211
Thick-bill    TTC ATC TGC ATT TAC CTC CAC ATT GGA CGA GGA TTC 211
Razorbill     TTC ATC TGT ATC TAC CTC CAC ATT GGA CGA GGA TTC 211
Dovekie       TTC ATC TGC ATC TAT CTC CAC ATC GGA CGA GGA TTC 211
Guillemot     TTC ATC TGC ATC TAC CTC CAC ATT GGA CGA GGA TTC 211
Puffin        TTC ATC TGC ATC TAC CTC CAC ATT GGA CGA GGA TTT 211
Gull          TTT ATT TGT ATT TAC CTA CAC ATC GGA CGA GGA TTC 211
Kittiwake     TTT ATC TGT ATC TAC TTA CAC ATT GGA CGA GGA TTC 211
Dunlin        TTC ATC TGC ATC TAC TTC CAC ATC GGA CGA GGC TTT 211
Plover        TTC ATC TGC ATC TAC CTC CAC ATC GGA CGA GGA TTT 211
Dove          TTC ATC TGT ATT TAC CTA CAC ATC GGA CGA GGA CTC 211

Common Murre  TAC TAC GGC TCA TAC CTG AAC                     232
Thick-bill    TAC TAC GGC TCG TAC CTA AAC                     232
Razorbill     TAC TAC GGC TCA TAC CTG AAC                     232
Dovekie       TAC TAC GGC TCA TAC CTA AAC                     232
Guillemot     TAC TAC GGC TCA TAC TTA AAC                     232
Puffin        TAC TAC GGT TCC TAC CTA AAC                     232
Gull          TAC TAT GGC TCA TAC CTC TAT                     232
Kittiwake     TAC TAC GGC TCC TAC CTC TAC                     232
Dunlin        TAC TAT GGC TCA TAT CTA TTC                     232
Plover        TAC TAT GGC TCC TAC CTA AAC                     232
Dove          TAC TAC GGA TCC TAC CTC TAC                     232
```

Fig. 5.1, Cont'd.

TABLE 5.4: Codon usage within a segment of the cytochrome *b* gene among 10 Charadriiform species and a rock dove.

Codon	Times Used	Codon	Times Used	Codon	Times Used
Alanine		**Glycine**		**Proline**	
GCA	41	GGA	62	CCA	10
GCC	41	GGC	34	CCC	0
GCG	1	GGG	2	CCG	0
GCT	6	GGT	8	CCT	0
Arginine		**Histidine**		**Serine**	
CGA	30	CAC	21	TCA	23
CGC	2	CAT	13	TCC	29
CGG	2			TCG	2
CGT	0	**Isoleucine**		TCT	1
		ATC	51	AGC	0
Aspartic Acid		ATT	24	AGT	0
GAC	8				
GAT	3	**Leucine**		**Threonine**	
		CTA	102	ACA	56
Asparagine		CTC	49	ACC	26
AAC	44	CTG	11	ACG	8
AAT	7	CTT	11	ACT	15
		TTA	12		
Cysteine		TTG	3	**Tryptophan**	
TGC	20			TGA	19
TGT	13	**Lysine**		TGG	2
		AAA	10		
Glutamic Acid		AAG	0	**Tyrosine**	
GAA	7			TAC	55
GAG	2	**Methionine**		TAT	23
		ATA	12		
Glutamine		ATG	3	**Valine**	
CAA	19			GTA	17
CAG	3	**Phenylalanine**		GTC	18
		TTC	62	GTG	2
		TTT	15	GTT	10

Common Murre				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Thick-bill				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Razorbill				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Dovekie				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Guillemot				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Puffin				phe	gly	ser	leu	leu	gly	ile	cys	leu	**thr**	10
Gull				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Kittiwake				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Dunlin				phe	gly	ser	leu	leu	gly	ile	cys	leu	**met**	10
Plover				phe	gly	ser	leu	leu	gly	ile	cys	leu	**ile**	10
Dove				phe	gly	ser	leu	leu	gly	ile	cys	leu	leu	10
Common Murre	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	thr	his		22
Thick-bill	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	thr	his		22
Razorbill	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	thr	his		22
Dovekie	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	thr	his		22
Guillemot	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	thr	his		22
Puffin	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**met**	his		22
Gull	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**met**	his		22
Kittiwake	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**met**	his		22
Dunlin	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**met**	his		22
Plover	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**met**	his		22
Dove	thr	glu	ile	leu	thr	gly	leu	leu	leu	ala	**ala**	his		22
Common Murre	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Thick-bill	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Razorbill	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Dovekie	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Guillemot	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Puffin	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Gull	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Kittiwake	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Dunlin	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Plover	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Dove	tyr	thr	ala	asp	thr	thr	leu	ala	phe	ser	ser	val		34
Common Murre	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Thick-bill	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Razorbill	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Dovekie	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Guillemot	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Puffin	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Gull	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Kittiwake	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Dunlin	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Plover	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46
Dove	ala	his	thr	cys	arg	asn	val	gln	tyr	gly	trp	leu		46

FIG. 5.2: Inferred amino acid sequences of a cytochrome *b* fragment for 10 Charadriiform species and a dove. Amino acid differences from the sequence of *Uria aalge* are highlighted.

Common Murre	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Thick-bill	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Razorbill	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Dovekie	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Guillemot	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Puffin	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Gull	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Kittiwake	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Dunlin	**leu**	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Plover	**leu**	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58
Dove	ile	arg	asn	leu	his	ala	asn	gly	ala	ser	phe	phe	58

Common Murre	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Thick-bill	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Razorbill	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Dovekie	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Guillemot	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Puffin	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Gull	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Kittiwake	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Dunlin	*phe*	*ile*	*cys*	*ile*	*tyr*	***phe***	*his*	*ile*	*gly*	*arg*	*gly*	*phe*	*70*
Plover	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	phe	70
Dove	phe	ile	cys	ile	tyr	leu	his	ile	gly	arg	gly	**leu**	70

Common Murre	tyr	tyr	gly	ser	tyr	leu	asn						77
Thick-bill	tyr	tyr	gly	ser	tyr	leu	asn						77
Dovekie	tyr	tyr	gly	ser	tyr	leu	asn						77
Guillemot	tyr	tyr	gly	ser	tyr	leu	asn						77
Puffin	tyr	tyr	gly	ser	tyr	leu	asn						77
Gull	tyr	tyr	gly	ser	tyr	leu	**tyr**						77
Kittiwake	tyr	tyr	gly	ser	tyr	leu	**tyr**						77
Dunlin	tyr	tyr	gly	ser	tyr	leu	**phe**						77
Plover	tyr	tyr	gly	ser	tyr	leu	asn						77
Dove	tyr	tyr	gly	ser	tyr	leu	**tyr**						77

Fig. 5.2. Cont'd.

ending in adenine were used 10.5 times more commonly than were those ending in guanine. Likewise, at two-fold degenerate sites involving pyrimidines, codons involving cytosine were used 3.5 times more frequently than those involving thymine. The larger bias between the two purines correlates with a greater molecular mass difference between these bases than between the pyrimidines. A less marked bias occurred in usage of purines *vs.* pyrimidines at four-fold degenerate sites: purines were utilized 1.5 times more frequently than pyrimidines. These patterns correlate with the fact that cytochrome *b* is encoded on the 'heavy' strand, and may be due to the energetics of codon-anticodon interactions (reviewed in Li *et al.* 1985). Brown (1983) also observed that the bias against guanines is strongest in homeotherms, and suggested it may result from intolerance of the tendency of guanines to form secondary structures in messenger RNA.

Thirty-six percent of nucleotide sites were variable among the 11 species examined (Fig. 5.1). Most variation occurred within third positions, and little variation occurred within second positions: 67 (86%) of 78 third positions varied, whereas only 14 (18%) of 77 and 2 (3%) of 77 first and second positions varied, respectively. Standard deviations in numbers of nucleotides at the three positions also indicated that third positions were the most variable, followed by first and then second positions (Table 5.3). These results agree with the fact that most first and all second position substitutions produce amino acid replacements in the mitochondrial genome, whereas third positions are more free to vary because of degeneracy of the genetic code. Substitutions within first positions also cause more conservative amino acid replacements than those in second positions (Brown 1985b). Most (10 of 14) first position substitutions in the present 11 species involved

leucines and did not result in amino acid replacements. Only 6 amino acids varied among the 11 species (Table 5.5; Fig. 5.2): three of these involved changes in functional groups, but none occurred at sites thought to be essential for function (Howell and Gilbert 1988, Kocher *et al.* 1989, Irwin *et al.* 1991). With the exception of the puffin *vs.* other alcids, no amino acid substitutions occurred among confamilial species.

Transitional substitutions were much more common than transversions at all taxonomic levels (Table 5.6; Fig. 5.1). A similar bias has been reported in many sequence analyses of mtDNA (e.g. Kocher *et al.* 1989, Edwards and Wilson 1990, Bartlett and Davidson 1991, Carr and Marshall 1991, Edwards *et al.* 1991, Irwin *et al.* 1991, McVeigh *et al.* 1991, Birt *et al.* 1992). This bias is probably due to the mutational process rather than to selection (Aquadro and Greenberg 1983). In the present data set, three sequences that differ by a single transversion are distinguished by 16—19 transitions ($\overline{X}$ = 17±1.7, Table 5.6). This is slightly lower than transition/transversion ratios of 20:1 in babblers (Edwards and Wilson 1990) and 24:1 in Amazon parrots (Birt *et al.* 1992), but slightly higher than a ratio of 12:1 for geese (Quinn *et al.* 1991).

PHYLOGENETIC ANALYSES

Fifty-nine phylogenetically informative sites were found within the 232 bp fragment of the cytochrome *b* gene among the present 11 species. Parsimony analysis did not resolve the alcids as a monophyletic group, and placed the shorebirds with the murres, razorbill and dovekie (cladogram not shown). This probably resulted from saturation effects: several researchers have reported that transversions and replacement substitutions accumulate with clock-like regularity, whereas transitions eventually plateau due to back-

TABLE 5.5: Total numbers of amino acid (above diagonal) and nucleotide (below diagonal) substitutions in a segment of the cytochrome *b* gene among 10 Charadriiform species and a rock dove.

	CM	TB	R	DK	BG	AP	HG	BK	DN	BP	RD
Common Murre	-	0	0	0	0	2	1	1	5	3	3
Thick-billed Murre	11	-	0	0	0	2	1	1	5	3	3
Razorbill	18	17	-	0	0	2	1	1	5	3	3
Dovekie	17	18	19	-	0	2	1	1	5	3	3
Guillemot	22	18	19	21	-	2	1	1	4	2	4
Puffin	28	27	29	31	22	-	1	1	4	2	4
Gull	34	28	26	28	34	31	-	0	4	2	2
Kittiwake	24	31	23	34	26	31	20	-	4	2	2
Dunlin	36	39	42	37	36	38	37	36	-	3	6
Plover	32	34	36	33	36	37	39	39	33	-	5
Dove	36	37	37	37	32	42	33	38	49	42	-

TABLE 5.6: Total numbers of transitions (above diagonal) and transversions (below diagonal) in a segment of the cytochrome *b* gene among 10 Charadriiform species and a rock dove.

	CM	TM	R	DK	BG	AP	HG	BK	DN	BP	RD
Common Murre	-	7	16	13	16	21	26	21	17	17	20
Thick-billed Murre	4	-	16	17	15	23	21	23	21	18	20
Razorbill	2	1	-	17	15	25	20	17	23	21	21
Dovekie	4	1	2	-	17	26	20	25	20	16	19
Guillemot	6	3	4	4	-	19	18	19	19	21	17
Puffin	7	4	5	5	3	-	24	25	22	25	20
Gull	8	7	6	8	6	7	-	19	22	24	23
Kittiwake	9	8	6	9	7	6	1	-	20	25	30
Dunlin	19	18	17	17	17	16	15	16	-	16	27
Plover	15	16	15	17	15	12	15	14	17	-	21
Dove	16	17	16	18	15	15	10	8	22	21	-

mutations (e.g. Avise *et al.* 1987a, Kocher *et al.* 1989, Irwin *et al.* 1991). Parsimony analyses that involved either weighting the relative values of transversions and transitions, or use of only transversions or replacement substitutions also resulted in unusual groupings of alcids with non-alcids (cladograms not shown). A plot (not shown) of number of transitions *vs.* number of transversions differentiating species pairs in Table 5.5 indicated that transitional substitutions become saturated above intra-familial comparisons. Phylogenetic analysis was therefore restricted to the Alcidae only, with the herring gull as an outgroup, using total substitutions. This analysis produced two equally parsimonious trees (total lengths = 80 steps; consistency indices excluding uninformative sites = 0.38; consistency indices including uninformative sites = 0.62). These trees differed only in grouping the razorbill with the murres *vs.* the dovekie. Bootstrap analysis produced the tree shown in Fig. 5.3. This tree is identical to one of the most parsimonious cladograms, and indicates the uncertainty in the placement of the razorbill. This ambiguity may result if the razorbill, dovekie and murres diverged within a relatively short time period.

The bootstrap tree for the Atlantic alcids agrees with the relationships proposed by Strauch (1985) and Sibley and Ahlquist (1990) in 1) clustering the dovekie sequence with the murre and razorbill, 2) clustering the guillemot outside the murres, razorbill and dovekie, and 3) placing the puffin sequence outside these four species. More sequence information may provide greater resolution of the relationships among these 7 species (Martin *et al.* 1990). The present results indicate the potential of direct sequencing of amplified DNA for elucidation of relationships among the alcids.

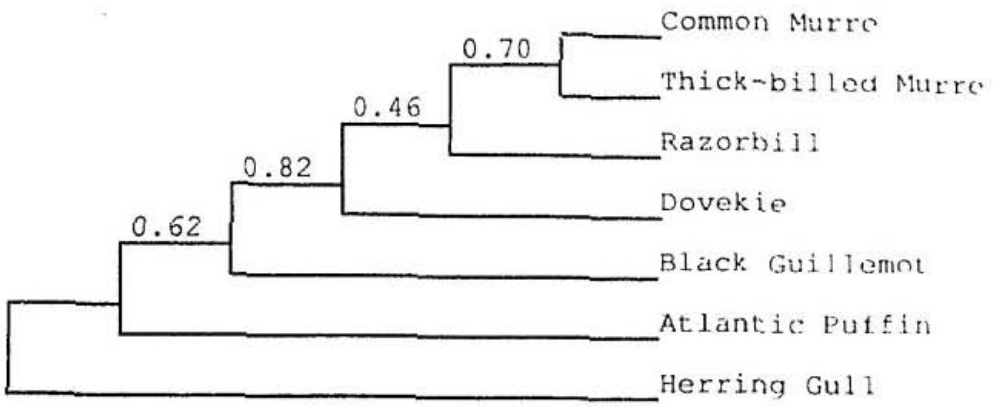

FIG. 5.3: Bootstrap analysis of the cytochrome *b* nucleotide sequences of the Atlantic alcids, rooted using a herring gull sequence. Numbers indicate the proportions of times that each association occurred during 1000 bootstrap replicates.

CHAPTER VI

GENERAL DISCUSSION

GENETIC DIFFERENTIATION WITHIN *VS.* AMONG THICK-BILLED MURRE COLONIES

In the present study, cytochrome *b* genotype frequencies were found to differ among thick-billed murres breeding at different sites within the colony at Hornøya, Norway (Chapter IV). This finding, in conjunction with evidence from band returns (Noble *et al.* 1991), morphometrics (A.J. Gaston *unpubl. data*), and egg and chick coloration (Gaston and Nettleship 1981, Birkhead 1985) suggests that thick-billed murres often breed on natal ledges, and that this intense philopatry can result in genetic differentiation within colonies.

Despite apparent substructuring within colonies, little genetic differentiation was found among five Atlantic colonies of thick-billed murres (Chapter II). These seemingly incompatible results may be explained by population dynamics and/or behaviour.

Population Dynamics.-Migration among colonies, at least of thick-billed murres, probably fits an island model, with exchange among colonies being independent of distance (Crow and Kimura 1970); frequencies of mtDNA genotypes will therefore be homogenized by exchange of only 2 females per generation (Birky *et al.* 1983). In contrast, migration among ledges more likely describes a two-dimensional stepping-stone model, with migration rate being a decreasing function of distance; homogenization of genotype

frequencies will therefore require migration rates 2—4 times higher than among colonies (Crow and Kimura 1970). Furthermore, effective population sizes will be much smaller within ledges than within colonies, so that genetic equilibrium between mutation and migration will be approached much more quickly among ledges (Mayr 1970, Slatkin and Maddison 1989). Genetic diversity may thus be predicted to be inversely related to ledge ages; unfortunately, ages of ledges were not known in the present study.

Behaviour.-Substructuring could also arise within colonies, despite genetic homogeneity among colonies, if low level gene flow occurs among colonies, but if recruits that return to their natal colonies always return to their natal ledges. This could involve an imperfect homing mechanism (Emlen 1975). Several seabird species have been found to exhibit this dispersal pattern: in Laysan albatrosses (Fisher 1971, 1976), herring gulls (Chabrzyk and Coulson 1976), northern gannets (Nelson 1978, R.T. Barrett *unpubl. data*, V.L.F. *unpubl. data*) and Atlantic puffins (Harris 1983), recruits often disperse, but those that return to natal colonies almost always nest close to natal sites. This type of homing would produce imperfect clustering of genotypes; i.e. birds within some breeding areas would be genetically similar, but genetic diversity would be greater within areas that had recently received immigrants. Areas with high diversity would probably be irregularly scattered among areas with low diversity. This pattern would be accentuated if some ledges were recently affected by either founder effects (e.g newly populated ledges in an expanding colony) or genetic bottlenecks (e.g. ledges recently subjected to rock falls or heavy predation). The observed pattern at Hornøya fits this predicted pattern (Table 4.5 [p. 64]); e.g. 91% of birds in area 2U possessed genotype 1, whereas area 2B had a broader

representation of genotypes. Inadvertant sampling of areas that either recently been colonized or recently received immigrants might explain the apparent absence of substructuring within Coats Island and Kipako.

PHENOTYPIC AND GENETIC DIFFERENTIATION AMONG ATLANTIC COLONIES OF THICK-BILLED *VS.* COMMON MURRES

In the present study, the extent of both morphological and genetic differentiation among Atlantic colonies was found to differ between thick-billed and common murres (Chapters II, III; Table 6.1). Specifically, the among-population component of phenotypic variation was twice as great for common murres, and G_{ST} for cytochrome *b* genotypes was more than an order of magnitude greater for common murres. These differences contrast with the biological similarities of the two species. Thick-billed and common murres are almost identical with respect to general breeding biology and demographics (Chapters I, III). Colonies of both species occur in areas that were glaciated during the Pleistocene, so are probably of similar ages. Furthermore, genetic diversities within colonies were similar between the species, which suggests that long-term effective population sizes and founder effects do not differ greatly between the species (Avise *et al.* 1988).

The contrasting genetic structures of common and thick-billed murres may result from differences in either gene flow or historical biogeography. Thick-billed murres breed continuously throughout the north Atlantic (Fig. 2.1 [p. 13]), whereas common murres do not nest between western Greenland and Iceland (Fig. 3.1 [p. 45]; Nettleship and Evans 1985). Also, most Atlantic thick-billed murres congregate off Greenland and Newfoundland

TABLE 6.1: Estimates of $\overline{F}_{ST}$ from morphometrics and protein electrophoresis, and G_{ST} for cytochrome *b* genotype frequencies among populations of thick-billed and common murres.

Population	Morphometrics	Nuclear Proteins	Cytochrome *b*
THICK-BILLED MURRES			
Atlantic/Pacific	-	-	0.30
East/West Atlantic	0.18	0.005	0.003
West Atlantic Colonies	0.18	0.023	0.004
COMMON MURRES			
Atlantic/Pacific	-	-	0.47
Atlantic Colonies	0.43	-	0.062

during winter (Gaston 1980, Kampp 1988, D.N. Nettleship *pers. comm.*), whereas common murres have several discrete wintering areas and rarely migrate across the Atlantic (Brown 1985a). Greater potential therefore exists for gene flow among colonies of thick-billed murres. Unfortunately, no comparative data are available at present to assess this possibility.

Pleistocene biogeographies may also have differed between the species. If modern wintering grounds of birds correspond to historical breeding grounds (Gauthreaux 1975), then modern colonies of common murres may have been founded from two or more genetically different refugial populations following the last glaciation, resulting in clines in cytochrome *b* genotypes and bridling (Chapter III). In contrast, modern thick-billed murre colonies may have been founded from one genetically homogeneous refugial population.

Assessment of the merits of these possibilities will require either extensive banding efforts or paleontological data. Further insight into the evolution of genetic structure in Atlantic alcids may also be gained by analyzing genetic structure in other species with different breeding distributions, wintering grounds and/or levels of gene flow. For example, Atlantic puffins breed continuously throughout the north Atlantic (Nettleship and Evans 1985), and British colonies appear to exchange recruits freely (Harris 1983). This suggests that, like thick-billed murres, puffins may exhibit little genetic differentiation among colonies. Nonetheless, at least two subspecies are described: 1) a large *naumanni* from Svalbard, Novaya Zemlya and eastern Greenland, and 2) a small *grabae* from the Atlantic (reviewed in Bédard 1985). Colonies of *grabae* appear not to differ genetically

(Moen 1991), but members of *naumanni* winter apart from the others, and may have survived the Pleistocene glaciations in northern refugia separate from other puffins (Glutz von Blotzheim and Bauer 1982 *in* Bédard 1985). If either Pleistocene breeding distributions or modern wintering grounds affect genetic structure, then members of *naumanni* should differ genetically from other puffins, including those at nearby colonies.

GENETIC DIFFERENTIATION BETWEEN ATLANTIC AND PACIFIC POPULATIONS OF THICK-BILLED AND COMMON MURRES

Analyses of cytochrome *b* genotype frequencies revealed that Atlantic and Pacific populations of both thick-billed and common murres are well differentiated genetically, and may represent incipient species (Chapters II, III; Table 6.1). This agrees with current separation of common murres in the two oceans by Arctic water and ice, and historical separation by the Bering Landbridge. Separation of Atlantic and Pacific thick-billed murres by the glaciations was probably less extensive due to their greater cold tolerance. Thick-billed murres also currently nest throughout the Arctic, so genetic differentiation between the Atlantic and Pacific should be less extensive than for common murres.

Evidence regarding the relative genetic differentiation of Atlantic and Pacific populations of the two species is ambiguous. G_{ST} for Atlantic *vs.* Pacific populations was slightly higher for common murres (0.47) than for thick-billed murres (0.30), which agrees with the expected pattern, but γ was similar for the two species (0.99 and 1.00 respectively). Similarly, no overlap was found between Atlantic and Pacific thick-billed murre genotypes (although Pacific sample sizes were small), whereas one common murre

genotype (UA4) occurred in both Atlantic and Pacific populations. This may suggest low level gene flow between oceans for common murres. However, UA4 differs from the major Atlantic and Pacific genotypes by a single silent transition each, so may have arisen independently in the two populations. It may also represent a retained ancestral polymorphism.

Resolution of the relative genetic isolation of Atlantic *vs.* Pacific populations of the two murres will require larger samples of both species from colonies spanning the Pacific. Samples from thick-billed murre colonies in the Arctic Ocean would also be interesting, since these colonies may have been populated either by northward dispersal from the Atlantic and/or Pacific (in which case the major Atlantic and/or Pacific genotypes should be present at some or all colonies), or from one or more glacial refugia within high Arctic polynyas (Flint 1971; in which case they should differ genetically from both Atlantic and Pacific thick-billed murres).

RECENT ZOOGEOGRAPHY OF COMMON MURRES

The substitutional networks for cytochrome *b* genotypes of the two murre species (Figs. 2.5 [p. 35], 3.2 [p. 51]) have several similarities. Specifically, both species possess one major genotype (UA1 and UL1) present in 68—75% of birds, and many 'satellite' genotypes that differ from the major type by only 1 or 2 transitions, mostly silent. However, the networks also have a curious difference: 21% of Atlantic common murres possess a secondary genotype (UA3) which differs from the major genotype by 5 nucleotide substitutions (p = 2.5%), including 2 amino acid substitutions. This genotype

is more than twice as different from UA1 as is the next most divergent genotype (p = 1.0%), and more than half as divergent from UA1 as is the most common thick-billed murre genotype (p = 4.4%). No genotypes intermediate between UA1 and UA3 were found. Wayne *et al.* (1990) reported a similar (but more extreme) scenario among black-backed jackals: specifically, the cytochrome *b* nucleotide sequence of one mtDNA genotype differed from all others by 14.5%.

Genotype frequency distributions may be explained by stochastic survival and extinction of mitochondrial lineages: i.e. genotypes UA1 and UA3 may have survived, and all intermediate genotypes have disappeared, by chance (genetic drift) alone. However, Avise *et al.* (1984b) argued that mtDNA genotypes that share recent ancestry should be most abundant, followed in declining frequency by more distantly related genotypes. The coexistence of two highly divergent genotypes is therefore improbable. The co-occurance of UA1 and UA3 may instead result from recent admixture of two separate ancestral populations. For example, the mitochondrial genomes of common murres may have diverged into an Atlantic lineage including UA3 and a Pacific lineage including UA1 or UA4 during an extended period of separation by the Bering Landbridge and/or Pleistocene glaciers (Flint 1971). Interglacial warming and opening of the Bering strait may have enabled subsequent invasion of the Atlantic by Pacific common murres, resulting in coexistence of the two lineages in the Atlantic. If this is correct, analysis of nuclear genes should reveal greater variation within Atlantic than Pacific common murres.

POPULATION DIFFERENTIATION AND SPECIATION IN THE GENUS *URIA*

The results of the present study suggest a general process of population differentiation and evolution within the genus *Uria*. Bédard (1985) proposed that the ancestral murre species diverged into a boreal *aalge* in the Pacific and a cold-water *lomvia* in the Arctic and North Atlantic Oceans during the Late Miocene or early Pliocene. Cytochrome *b* nucleotide sequences and allozyme data suggest this occurred ~2.4—4.1 mya (Chapter V). During the early Quaternary, common murres may have invaded the Atlantic, and thick-billed murres may have invaded the Pacific. Subsequently, Atlantic and Pacific common murres may have been separated for a million years or more by the Bering Landbridge and/or glacial ice sheets. During this time, their mitochondrial genomes may have diverged into a Pacific lineage involving genotypes UA1 and/or UA4, and an Atlantic lineage including genotype UA3. Bridling may also have evolved in the Atlantic at this time. Interglacial warming may have allowed a second Atlantic invasion by Pacific common murres and mixing of the two mitochondrial lineages. More recent cooling may have separated the populations again and forced Atlantic common murres into two main refugial centres: 1) a northeastern centre consisting mostly of murres with bridles and genotype UA1 and its derivatives, and 2) a more southerly centre consisting of a high proportion of murres with genotype UA3 and lacking bridles. In the last 10,000—20,000 years, murres may have dispersed gradually from these refugial centres throughout the Atlantic, producing north-south clines in cytochrome *b* genotype frequencies and bridling (Chapter III; Southern 1962, Birkhead 1984).

Although Atlantic and Pacific thick-billed murres probably were not separated to the same extent as were common murres during most of the Pleistocene, the two populations of thick-billed murres were probably separated during the last glaciations. At that time their mitochondrial genomes diverged into a Pacific lineage including genotype UL16 and an Atlantic lineage involving genotype UL1. Atlantic thick-billed murres may have survived this glaciation in one main refugial centre, from which modern colonies were populated within the last 10,000 years.

Since the last glaciations, strong natal philopatry has prevented phenotypic homogenization of Atlantic colonies of both species, as well as genetic panmixia among common murre colonies (Chapters II, III; Storer 1952, Birkhead and Nettleship 1981, Gaston *et al.* 1984, Bédard 1985). Homing to natal ledges in both species (Noble *et al.* 1991) has also lead to phenotypic and genetic substructuring within some colonies (Chapter IV; Birkhead *et al.* 1980, Gaston and Nettleship 1981, Birkhead 1985, A.J. Gaston *unpubl. data*), and may facilitate the evolution of altruistic behaviour (Tuck 1960, Birkhead 1985).

COMPARISONS WITH OTHER STUDIES

A large number of studies of population genetic differentiation have accumulated since the advent of electrophoresis (avian studies reviewed in Barrowclough 1983, Corbin 1987, Evans 1987, Barrowclough and Johnson 1988). Studies involving protein electrophoresis suggest that, although genic variation (average heterozygosity, proportion of loci polymorphic) in birds is as great as in other vertebrates, population genetic

differentiation is generally very low. Barrowclough and Johnson (1988) interpreted these findings as indicative of moderate effective population sizes and/or significant gene flow in most bird populations compared to most other vertebrates. Data from protein electrophoresis (Ayala 1976, Avise and Aquadro 1982, Corbin 1987, Evans 1987), cytogenetics, and interspecific hybridizations (Prager and Wilson 1975) also suggest much lower genetic distances among congeneric species of birds than among other vertebrate species. These results have been interpreted to indicate more recent speciation events in birds and/or slower rates of protein evolution (Avise and Aquadro 1982).

Explanation of the apparent differences in evolutionary rates between birds and other vertebrates has awaited an independent metric of genetic variation. Enough mtDNA studies of intra- and inter-specific differentiation are now available for preliminary comparisons with data from protein electrophoresis (Tables 6.2-6.6).[1] The species in these studies can be classified into five general catagories based on dispersal capabilites: 1) aerial species (birds and bats), 2) marine and catadromous species, 3) anadromous fishes, 4) freshwater fishes, and 5) terrestrial species (most mammals, reptiles and amphibians). ANOVA indicated significant differences in population genetic differentiation and nucleotide divergence among these groups, with differentiation being generally greater in freshwater and terrestrial species than in aerial and marine species (Tables 6.5, 6.6). This agrees with general dispersal capabilities: mammals, especially females, tend not to

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1) Unfortunately, not all papers enable calculation of G_{ST} due to either data presentation or small sample sizes. G_{ST} and γ also do not account for sequence divergence among genotypes, and may thus underestimate the genetic divergence among distantly related populations. Furthermore, sampling sites in many studies do not encompass the species' entire range. Nonetheless, these studies enable a general picture of patterns of population and species differentiation.

TABLE 6.2: Inter-population components of genetic variation (G_{ST} and γ) calculated from published studies involving mtDNA analyses. (Local populations with < 4 samples were excluded from calculations, hence sample sizes may differ from those in Table 6.3.)

Species	Dispersal Class[1]	G_{ST}	γ	Number			Protocol[2]	Reference
				Individuals	Demes	Genotypes		
INVERTEBRATES								
Drosophila sulfurigaster albostrigata[3]	0	0.44	0.55	52	4	6	R	Tamura *et al.* 1991
D. s. bilimbata[4]	0	0.50	0.88	56	6	11	R	Tamura *et al.* 1991
Magicicada tredecim	0	0.63	0.55	80	11	10	R	Martin and Simon 1990
Strongylocentrotus droebachiensis	0	0.46	0.56	41	3	6	R	Palumbi and Wilson 1990
S. purpuratus	0	0.00	0.00	38	2	20	R	Palumbi and Wilson 1990
FISH								
Alosa sapidissima[5]	3	0.12	0.32	204	13	15	R	Bentzen *et al.* 1989
Anguilla anguilla	2	0.01	0.01	67	2	20	R	Avise *et al.* 1986
A. rostrata	2	0.03	0.32	109	7	26	R	Avise *et al.* 1986
Fundulus heteroclitus	2	0.25	0.76	48	4	17	R	Gonzaléz-Villaseñor and Powers 1990
Gadopsis bispinosus	4	1.00	0.67	12	3	2	R	Ovenden *et al.* 1988
G. marmoratus	4	0.66	0.81	27	6	6	R	Ovenden *et al.* 1988
Gadus morhua	2	0.12	0.13	54	5	12	C	Carr and Marshall 1991
Galaxia truttaceus[6]	2	0.01	0.31	141	9	56	R	Ovenden and White 1990
Gasterosteus aculeatus	4	0.27	0.42	30	4	7	R	Gach and Reimchen 1989

TABLE 6.2, Cont'd.

Species	Dispersal Class[1]	G_{ST}	γ	Number			Protocol[2]	Reference
				Individuals	Demes	Genotypes		
FISH, Cont'd								
Hoplostethus atlanticus	2	0.02	0.02	49	2	11	R	Ovenden *et al.* 1989
Lepomis macrochirus[7]	4	1.00	0.67	24	4	2	R	Avise *et al.* 1984a
L. punctatus	2	0.50	0.71	65	10	16	R	Bermingham and Avise 1986
Morone americana	3	0.58	0.24	254	8	10	R	Mulligan and Chapman 1989
M. saxatilis[8]	3	0.11	0.13	110	7	5	R	Chapman 1990
Salmo salar	3	0.43	0.40	30	7	3	R	McVeigh *et al.* 1991
REPTILES								
Chelonia mydas	2	0.92	0.98	46	5	12	R	Bowan *et al.* 1989
Xerobates agassizi	5	0.87	0.46	29	7	3	R	Lamb *et al.* 1989
BIRDS								
Ammospiza maritima	1	0.40	0.62	39	5[8]	11	R	Avise and Nelson 1989
Anas platyrhynchos	1	0.00	0.00	20	2	7	R	Avise *et al.* 1990
A. rubripes	1	0.05	0.08	20	2	3	R	Avise *et al.* 1990
Branta canadensis	1	0.90	1.00	43	5	8	R	Van Wagner and Baker 1990
Melospiza melodia	1	0.00	0.00	22	5	11	R	Zink 1991
Molothrus ater	1	0.11	0.11	69	7	2	R	Fleischer *et al.* 1991
Pachyptila turtur	1	0.16	0.25	61	4	15	R	Ovenden *et al.* 1991
Passerella iliaca	1	0.48	0.51	89	9	5	R	Zink 1991
Pomatostomus temporalis[9]	1	0.15	0.77	34	4	15	C&S	Edwards and Wilson 1990
Quiscalus quiscula	1	0.01	0.54	29	6	25	R	Zink *et al.* 1991a

TABLE 6.2 Cont'd.

Species	Dispersal class[1]	G_{ST}	γ	Number			Protocol[2]	Reference
				Individuals	Demes	Genotypes		
BIRDS, Cont'd								
Uria aalge, Holarctic	1	0.37	0.44	142	5	10	C	*Present study*
Atlantic	1	0.06	0.06	130	4	8	C	*Present study*
Norwegian	1	0.00	0.00	51	4	13	R	Moum *et al.* 1991
U. lomvia, Holarctic	1	0.22	0.34	239	6	18	C	*Present study*
Atlantic	1	0.00	0.01	235	5	16	C	*Present study*
MAMMALS								
Apodemus sylvaticus	5	0.20	0.43	44	5	7	R	Byrne *et al.* 1990
Artibeus jamaicensis	1	0.65	0.60	54	3	3	R	Pumo *et al.* 1988
Canis mesomelas	5	0.13	0.26	60	4	4	R	Wayne *et al.* 1990
Dipodomys panamintus	5	0.31	0.98	106	3	24	D	Thomas *et al.* 1990
Macaca fuscata	5	0.74	0.62	94	8	4	S	Hayasaka *et al.* 1991
Microtus pennsylvanicus	5	0.38	1.00	99	7	37	R	Plante *et al.* 1989b
Nycticeius humeralis[5]	1	0.13	0.11	140	7	3	S	Wilkinson & Chapman 1991
Odocoileus hemionus	5	0.60	0.89	48	8	9	C	Carr and Hughes 1992; Hughes and Carr 1992
O. hemionus[10]	5	1.00	1.00	24	2	2	R	Carr *et al.* 1986
O. virginius[10]	5	0.81	0.90	66	5	5	R	Carr *et al.* 1986
Onychomys leucogaster	5	0.66	0.95	43	5	29	R	Riddle and Honeycutt 1990
Orcinus orca	2	0.58	1.00	19	2	3	R	Stevens *et al.* 1989
Peromyscus maniculatus	5	0.59	0.81	123	11	23	R	Ashley and Wills 1987
Spermophilus columbianus	5	1.00	0.60	39	6	2	R	MacNeil and Strobeck 1987

Table 6.2, Cont'd.

1) 0 = invertebrate; 1 = aerial; 2 = marine or catadromous; 3 = anadromous; 4 = freshwater; 5 = terrestrial.
2) C = Cytochrome *b* nucleotide sequence; D = D-loop nucleotide sequence; R = RFLP analysis; S = size polymorphism.
3) Mainland Asia.
4) Insular Asia.
5) Excluding heteroplasmic individuals.
6) Catadromous populations only.
7) Two subspecies.
8) Excluding 3+ year males; spawning populations only.
9) Five subspecies.
10) Excluding interspecific hybrids.

TABLE 6.3: Mean percent sequence divergence ($\bar{p}$) among mtDNA RFLP genotypes of vertebrate species.

Species	Dispersal Class[1]	Number			$\bar{p}$	Reference
		Individuals	Sites	Genotypes		
INVERTEBRATES						
Drosophila sulfurigaster	0	108	19	17	7.59	Tamura *et al.* 1991
Strongylocentrotus purpuratus	0	38	3	6	0.48	Palumbi and Wilson 1990
S. droebachiensis	0	41	3	6	0.10	Palumbi and Wilson 1990
FISH						
Alosa sapidissima	3	52	14	13	0.37	Bentzen *et al.* 1989
Amia calva	4	75	20	13	0.78	Bermingham and Avise 1986
Clupea harengus	2	69	3	26	1.66	Kornfield and Bogdanowicz 1987
Gadopsis marmoratus	4	38	12	7	5.98	Ovenden *et al.* 1988
G. bispinosus	4	12	3	2	8.85	Ovenden *et al.* 1988
Galaxia truttaceus	2	211	17	58	0.88	Ovenden and White 1990
Hoplostethus atlanticus	2	49	2	11	0.59	Ovenden *et al.* 1989
Lepomis gulosus	4	17	74	32	4.80	Bermingham and Avise 1986
L. microlophus	4	77	17	7	4.75	Bermingham and Avise 1986
L. punctatus	4	79	16	17	4.82	Bermingham and Avise 1986
Merluccius capensis	2	26	10	14	0.60	Becker *et al.* 1988
M. punctatus	2	24	8	6	0.50	Becker *et al.* 1988
Oncorhynchus tshawytscha	3	76	7	6	0.48	Wilson *et al.* 1987
Salmo gairdneri[2]	3	38	8	8	0.66	Wilson *et al.* 1985b

TABLE 6.3, Cont'd.

Species	Dispersal Class[1]	Number			$\bar{p}$	Reference
		Individuals	Demes	Genotypes		
REPTILES						
Xerobates agassizi	5	57	22	5	3.67	Lamb *et al.* 1989
BIRDS						
Agelaius phoeniceus	1	127	19	34	0.2	Ball *et al.* 1989
Branta canadensis[3]	1	53	9	14	1.39	Van Wagner and Baker 1990
Branta canadensis[4]	1	8	8	8	0.71	Shields and Wilson 1987a, b, Quinn *et al.* 1991
Melospiza melodia	1	27	7	15	0.27	Zink 1991
Passerella iliaca	1	46	9	5	0.60	Zink 1991
Platycercus elegans[5]	1	8	6	7	0.66	Ovenden *et al.* 1987
P. adscitus[5]	1	7	6	6	0.27	Ovenden *et al.* 1987
Quiscalus quiscula	1	35	8	29	0.28	Zink *et al.* 1991a
Uria aalge[6]	1	51	4	13	0.31	Moum *et al.* 1991

TABLE 6.3, Cont'd.

Species	Dispersal Class[1]	Number			$\bar{p}$	Reference
		Individuals	Demes	Genotypes		
MAMMALS						
Artebeus jamaicensis	1	54	3	3	5.9	Pumo *et al.* 1988
Canis mesomelas	5	64	6	4	4.5	Wayne *et al.* 1990
Mus domesticus[7]	5	82	21	4	0.77	Ferris *et al.* 1983
Odocoileus hemionus[8]	5	25	2	2	5.8	Carr *et al.* 1986
O. virginianus[8]	5	54	4	5	0.8	Carr *et al.* 1986
Onychomys arenicola	5	8	8	3	1.4	Riddle and Honeycutt 1990
O. leucogaster[9]	5	44	6	30	2.58	Riddle and Honeycutt 1990
O. torridus	5	12	12	9	0.7	Riddle and Honeycutt 1990
Pan troglodytes	5	69	4	5	1.3	Ferris *et al.* 1981
Peromyscus leucopus	5	18	25	55	0.54	Nelson *et al.* 1987
Spermophilus columbianus	5	71	12	4	0.43	MacNeil and Strobeck 1987

1) 0 = Invertebrate; 1 = aerial; 2 = marine or catadromous; 3 = anadromous; 4 = freshwater; 5 = terrestrial.
2) Both anadromous and landlocked populations.
3) Five subspecies.
4) Eight subspecies.
5) Four subspecies.
6) Four Norwegian colonies.
7) 21 wild populations plus inbred laboratory strains.
8) Excluding interspecific hybrids.
9) Mean of means for three geographic areas.

TABLE 6.4: Mean percent sequence divergence ($\bar{p}$) among mtDNA genotypes of congeneric species analyzed using restriction endonucleases. Interspecific values averaged across intraspecific genotypes where applicable.

Genus	Dispersal Class[1]	Species Analyzed	$\bar{p}$	Reference
INVERTEBRATES				
Drosophila	0	8	6.3	Solignac *et al.* 1986
Heterodera	0	2	14.5	Radice *et al.* 1988
Strongylocentrotus	0	2	6.0	Palumbi and Wilson 1990
FISH				
Anguilla	2	2	3.7	Avise *et al.* 1984a
Coreogonus	3	2	0.9	Bernatchez *et al.* 1988
Gadopsis	4	2	14.0	Ovenden *et al.* 1988
Lepomis	4	9	25.7	Kessler and Avise 1984a
Merluccius	2	2	11.6	Becker *et al.* 1988
Notropis	2	4	9.9	Dowling and Brown 1989
Oncorhynchus	3	2	6.4	Gyllenstein and Wilson 1987
Opsanus	2	2	10.0	Avise *et al.* 1987b
Salmo	3	2	6.4	Gyllentsein and Wilson 1987
Salvelinus	3	5	2.9	Grewe *et al.* 1990
AMPHIBIANS AND REPTILES				
Bombina	5	2	9.4	Szymura *et al.* 1985
Gopherus	5	2	4.5	Lamb *et al.* 1989
Hyla	5	5	26.0	Kessler and Avise 1984a
Xenopus	5	7	24.5	Carr *et al.* 1987
Xerobates	5	2	6.4	Lamb *et al.* 1989
BIRDS				
Ammodramus	1	8	7.3	Zink and Avise 1990
Anas	1	9	6.2	Kessler and Avise 1984a, b
Anser	1	3	2.1	Shields and Wilson 1987b
Aythya	1	4	3.4	Kessler and Avise 1984a,b
Branta	1	2	6.1	Quinn *et al.* 1991
Calidris	1	4	9.9	Dittmann and Zink 1991
Dendroica	1	5	4.4	Kessler and Avise 1984a
Limnodramus	1	2	8.2	Avise and Zink 1988
Papilo	1	5	6.1	Zink and Dittmann 1991
Parus	1	3	7.3	Mack *et al.* 1986
Platycercus	1	6	4.6	Ovenden *et al.* 1987
Quiscalus	1	4	3.2	Zink *et al.* 1991a
Rallus	1	2	0.6	Avise and Zink 1988
Zonotrichia	1	5	2.9	Zink *et al.* 1991b

TABLE 6.4, Cont'd.

Genus	Dispersal Class[1]	Species Analyzed	$\bar{p}$	Reference
MAMMALS				
Apodemus	5	2	10.0	Tegelström *et al.* 1988
Bathyergus	5	2	7.6	Honeycutt *et al.* 1987
Canis	5	3	10.6	Wayne *et al.*1990
Cleithrionomys	5	2	13.9	Tegelström *et al.* 1988
Equus	5	6	6.3	George and Ryder 1986
Microtus	5	2	6.3	Plante *et al.* 1989b
Mus	5	6	5.7	Ferris *et al.* 1983
Odocoileus	5	2	3.5	Carr *et al.* 1986
Onychomys	5	3	6.6	Riddle and Honeycutt 1990
Pan	5	2	3.7	Ferris *et al.* 1981
Peromyscus	5	3	10.8	Avise *et al.* 1991
Rattus	5	2	16.0	Brown and Simpson 1981
Spermophilus	5	3	5.7	MacNeil and Strobeck 1987

1) 0 = invertebrate; 1 = aerial; 2 = marine or catadromous; 3 = anadromous; 4 = freshwater; 5 = terrestrial.

TABLE 6.5: Comparison of genetic divergences within and among vertebrate species that differ in dispersal modes[1]. Data from Tables 6.2—6.4. Means with different letter superscripts differ according to Tukey's studentized range test.

Class		G_{ST}	γ	Intra-specific $\bar{p}$	Inter-specific $\bar{p}$
Aerial	*X*	0.24[a]	0.42[a]	1.10[a]	5.12[a]
	SD	(0.27)	(0.34)	(1.84)	(4.83)
	N	15	15	9	16
Marine/	*X*	0.21[a]	0.45	0.64[a]	8.80[ab]
Catadromous	*SD*	(0.27)	(0.40)	(0.17)	(2.55)
	N	8	8	4	4
Anadromous	*X*	0.31	0.27	0.50[a]	3.58[ac]
	SD	(0.23)	(0.12)	(0.15)	(3.49)
	N	4	4	3	4
Freshwater	*X*	0.64[b]	0.71	5.00[b]	19.85[b]
	SD	(0.31)	(0.18)	(2.60)	(8.27)
	N	6	6	6	2
Terrestrial	*X*	0.62[b]	0.74[b]	2.05	9.96[bc]
	SD	(0.30)	(0.26)	(1.84)	(6.48)
	N	12	12	11	18
F		5.52	2.73	5.93	6.25
P		0.001	0.04	0.001	0.001

1) ANOVA, arcsine (square root) transformed data.

TABLE 6.6: Comparisons of variation in proteins and mtDNA in birds with other vertebrates. See text and Tables 6.2—6.4 for references.

Anatomy	Interspecific *H*	Birds = mammals > other vertebrates
Proteins	Genic variation	Birds = other vertebrates
	Population differentiation	Birds < other vertebrates
	Interspecific differentiation	Birds < other vertebrates
MtDNA	Population differentiation	Aerial, marine < freshwater, terrestrial
	% sequence divergence	
	Within species	Aerial, marine < freshwater, terrestrial
	Between species	Aerial, anadromous < freshwater, terrestrial

disperse far from natal areas, and freshwater fish cannot migrate between rivers, but most birds and marine animals are highly mobile. Nucleotide divergence among congeneric species also appears to be greater in species with limited dispersal capabilities (Tables 6.4, 6.5). The tendency for species with low dispersal capabilities to exhibit greater genetic differentiation also holds on a qualitative basis within birds; the few avian populations that exhibit moderate to strong genetic differentiation are characterized by historical and/or modern barriers to dispersal (Atlantic *vs*. Pacific murres, grey-crowned babblers, fox sparrows and seaside sparrows *Ammospiza maritima*) or strong natal philopatry (fairy prions, Canada geese).

MtDNA results thus corroborate results for protein electrophoresis, which indicate little genetic differentiation within and among bird species compared with mammals and freshwater fishes (Table 6.6). However, genetic differentiation in birds does not appear unusual in comparison with marine species, and the rate of mtDNA evolution in birds appears to be similar to other vertebrates (Shields and Wilson 1987b). The low genetic differentiation within and among avian species therefore probably results from high levels of homogenizing gene flow resulting from high dispersal capabilities. High dispersal capabilites will result in greater effective population sizes, which will maintain genic diversity within species and reduce genetic divergence among species.

Despite the low divergence within and among avian species, rates of anatomical evolution and speciation appear to be at least as high as in other vertebrates (Prager and Wilson 1975, Wyles *et al.* 1983; but see also Hafner *et al.* 1984, Wilson *et al.* 1984). Thus, despite their high dispersal capabilites, birds must frequently encounter barriers to

dispersal that result in sufficient genetic isolation of populations for speciation to occur.

CONSERVATION RECOMMENDATIONS

Indirect and direct evidence indicates that genetic substructuring exists within some (if not most) colonies of murres. Loss or decimation of part of a colony, e.g. through egging, may therefore significantly reduce a colony's genetic resources. Similarly, clinal variation in both cytochrome *b* genotype frequencies and bridling in Atlantic common murres indicates that genetic differences exist among these colonies. Loss or decimation of individual colonies (e.g. due to chronic pollution or oil spills) would therefore reduce the genetic resources of the species, and may thus affect its ability to adapt to environmental change (Allendorf and Leary 1986, Gilpin and Soulé 1986). The lack of genetic differentiation in nuclear proteins and mtDNA among Atlantic thick-billed murres suggests that loss of individual colonies may have less affect on the genetic resources of this species compared with common murres; nonetheless, morphological differences among colonies suggest that important genetic differences may not have been detected. Furthermore, gene flow cannot be estimated using the present techniques, and if gene flow is restricted, recovery of colonies from local extinctions may be slow or nil. For example, common murres have failed to repopulate several colonies on Quebec's south shore from which they were eliminated by egging in the early 1900s (Nettleship and Evans 1985). Therefore, until more evidence from band returns is available, it should be assumed that gene flow among colonies of both thick-billed and common murres is negligible, and that murres may not repopulate areas from which they are extirpated (see

also Cairns and Elliot 1987). It is therefore advisable that representative colonies spanning the entire Atlantic ranges of both species be protected, and that conservation policies apply to whole colonies.

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