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MITOCHONDRIAL DNA LINEAGES IN COMPOSITE FLOCKS OF MIGRATORY AND WINTERING DUNLINS (*CALIDRIS ALPINA*)

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ABSTRACT.—Mitochondrial DNA (mtDNA) control-region sequences of 52 migratory and wintering Dunlins (*Calidris alpina*) from around the world were determined with direct sequencing of PCR products. The genetic lineages detected in these birds are identical to those found previously in a much larger sample of 155 breeding Dunlins from their northern circumpolar range. Samples of nonbreeding Dunlins from both sides of the Pacific reveal a mixture of two lineages that breed separately in eastern Siberia and Alaska. The presence of Dunlins with an eastern Siberian haplotype along the west coast of North America indicates that the Bering Strait does not represent a biogeographic barrier to Dunlin migration. Dunlins wintering in eastern Asia most likely originated from the discrete breeding population in northern Alaska because they possess haplotypes that were found predominantly in birds from this region. Similarly, Dunlins from staging and wintering sites in Europe and western Asia reveal a mixture of two mtDNA lineages that were previously found confined largely to European and central Siberian breeding grounds. Limited gene flow between these breeding areas, however, precludes definitive allocation of individuals to their population of origin on the basis of mtDNA analysis alone. Body mass, time of migration, and molting pattern seem to be associated with the mtDNA types of migratory Dunlins in Europe, but data are too sparse to determine whether these characters are useful adjuncts in assigning nonbreeding birds to populations that correspond to the major genetic lineages. Overall, the genetic composition of nonbreeding populations indicates the confluence of breeding populations on southward migration. Because of strong phylogeographic population structure in Dunlins on their breeding grounds, mtDNA analysis can be extremely useful in defining broad migration corridors or flyways, and in determining staging and wintering areas used by the major breeding populations. *Received 6 June 1994, accepted 27 January 1995.*

MANY SHOREBIRD SPECIES breed on the arctic tundra during the short polar summer. In the rest of the year these shorebirds winter in temperate to tropical latitudes, or are on migration between the two distant habitats. Several wide migration corridors, referred to as flyways, are used by shorebirds around the world. One of the more abundant migrants of the Northern Hemisphere is the Dunlin (*Calidris alpina*), which has an almost circumpolar distribution of breeding populations. Although no absolute separation occurs between the populations of Dunlins that use these flyways, a broad distinction into five migratory groups can be made. These groups use the West Atlantic, East Atlantic, Mid-Eurasian, West Pacific, and East Pacific migration flyways (Davidson and Pienkowski 1987).

Unlike most other shorebirds, the Dunlin shows considerable phenotypic variation over its range. Six morphometrically differentiated populations have been distinguished in a worldwide analysis, with bill size accounting for 85% of all measured variation (Greenwood 1986). Greenwood examined breeding populations because band recoveries already had shown extensive mixing of populations on the wintering grounds (Greenwood 1984). Morphometrics have limited use for the assignment of individual Dunlins to populations because character means of birds from different breeding populations overlap substantially. Pronounced sexual dimorphism in size for the otherwise very similar sexes adds to this problem. Knowledge of the population composition of migratory and wintering flocks is not only extremely valuable in assessing the status of populations, but also is relevant to the preservation of genetic diversity within species (Avise 1989, Avise and Nelson 1989).

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We have demonstrated previously the utility of mitochondrial DNA (mtDNA) in revealing population-genetic as well as evolutionary aspects of intraspecific differentiation in Dunlins (Wenink et al. 1993, 1994). The suitability of mtDNA for this purpose rests largely on its maternal inheritance, almost complete lack of recombination, and high speed of mutation (reviewed in Wilson et al. 1985, Avise et al. 1987). Sequence analysis of the most variable part of the Dunlin mtDNA genome, the noncoding control region, enables the probing of historical divergence of populations as recently as 10,000 years ago. Globally, five major mtDNA lineages were detected in Dunlins; these lineages all have a strong geographic specificity over the species' breeding range. This phylogeographic pattern likely originated during the late Pleistocene, between approximately 70,000 and 230,000 years ago, as a result of habitat fragmentation by successive glaciations. The resultant subdivided population structure must have been retained after retreat of the ice sheets by strong natal homing of Dunlins to their breeding ground (Wenink et al. 1993, 1996).

The five flyways that Dunlins currently use may reflect the post-Pleistocene expansion routes of these historically isolated populations. The approximate distribution of the five mtDNA phylogeographic groups or major populations on the breeding grounds is indicated in Figure 1. Each group has been labeled according to its geographic location, and also coincides with a morphometrically defined subspecies as follows: group I labeled as Alaska (*C. a. pacifica*); group II as eastern Siberia (*C. a. sakhalina*); group III as central Siberia (*C. a. centralis*); group IV as Europe (*C. a. alpina*); and group V as Canada (*C. a. hudsonia*; Wenink et al. 1996). Limited gene flow was observed between the central Siberian group and the flanking groups in Europe and eastern Siberia.

Bearing in mind the phylogeographic population structure of Dunlins on the breeding grounds, we here assess the potential of mtDNA sequence analysis in revealing the distribution of Dunlin populations outside their breeding range. We show that mtDNA control-region sequences diagnostic of major breeding populations around the world are of great utility in tracking migrants along flyways and in determining which flyways are used by populations. When simplified to the presence or absence of a restriction site in the control-region segment, this technique can be used to determine the provenance of birds in composite winter flocks in Europe and, thus, should prove to be invaluable in the conservation of rarer populations.

TABLE 1. Collection locales and sample sizes of Dunlins analyzed.

Locale	*n*	Abbreviation
1 Wadden Sea; Germany and The Netherlands	16	WAD
2 Gdansk, Poland	8	GDA
3 Krym, Ukraine	6	KRY
4 Persian Gulf; Bahrain and Saudi Arabia	8	GUL
5 Hong Kong	4	HON
6 Kamchatka Peninsula, Russia	1	KAM
7 Washington, USA	4	WAS
8 California, USA	5	CAF

Materials and Methods

Sample details.—All samples were collected as a few drops of blood taken from the major wing vein and were immediately mixed with 50 mM EDTA and stored in 70% ethanol at 4°C (or at room temperature), or were snap-frozen as solid tissue in liquid nitrogen and stored in the laboratory at −70°C (for birds from Washington and California). Details of samples are given in Table 1, and collection locales of staging or wintering Dunlins are indicated in Figure 1. Sequences of six birds from the Wadden Sea in Europe and nine birds from the west coast of North America have been reported previously (Wenink et al. 1993) and also are employed here.

Amplification, sequencing, and restriction analysis.—Total DNA was isolated from approximately 25 μL of blood or 10 mg of tissue according to standard procedures (Sambrook et al. 1989). Amplification of the hypervariable control-region segments I and II was conducted with primers and conditions detailed in Wenink et al. (1993). An M13-forward sequence was added to the 5' end of each heavy strand primer to facilitate Dye-primer labeling. Amplification products were sequenced directly using four fluorescent Dye-primers (Applied Biosystems) and *Taq* DNA polymerase in a cycle sequencing protocol. This protocol consisted of 15 cycles of 30 s at 95°C, 30 s at 55°C, 60 s at 70°C, followed by 15 cycles of 30 s at 95°C and 60 s at 70°C. The labeled products were collected by ethanol precipitation and separated on an 8% polyacrylamide/7M Urea gel in an ABI 373A automated DNA sequencer according to the manufacturer's instructions. Sequences were aligned with the on-line Sequence Editor program.

To test whether it was possible to develop a rapid

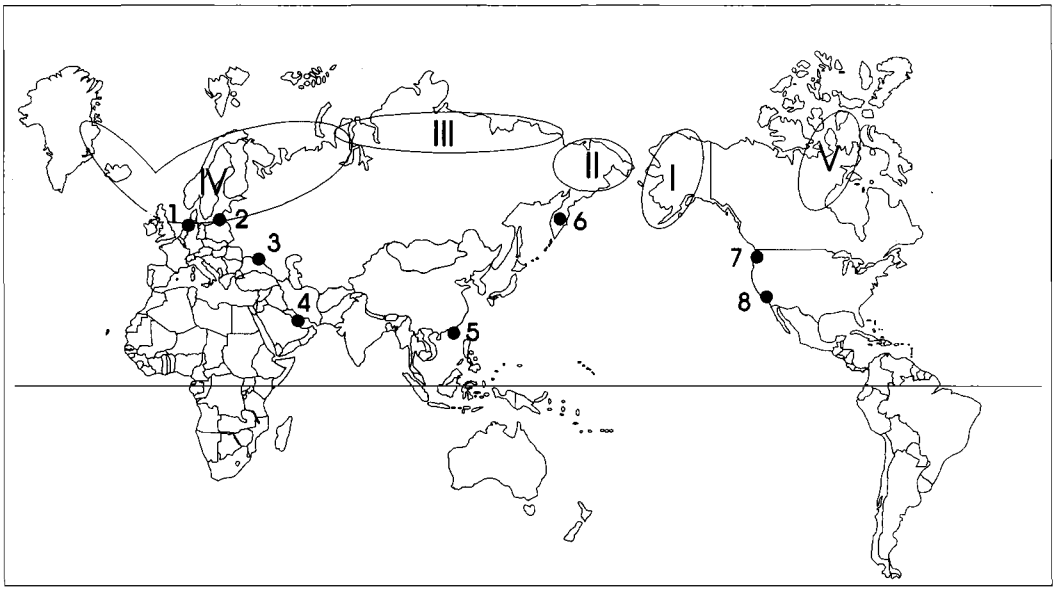

FIG. 1. Map showing sample locales (1–8) of Dunlins caught during migration or winter. Sample sizes and locale details given in Table 1. Roman numbers (I–V) indicate the five phylogeographic groups of Dunlins that were identified previously on the breeding grounds (Wenink et al. 1996).

and relatively inexpensive method to type individuals, we searched both control-region segments using MICROGENIE (Beckmann Instruments) for the presence or absence of restriction sites diagnostic of the two mtDNA lineages that mix at wintering sites in Europe. The test was restricted to Europe because major populations attributable to different mtDNA lineages are known to mix there during staging or at wintering sites. One-quarter of the precipitated control region I DNA amplification product was incubated for 3 h with *Alu I* restriction enzyme (Boehringer Mannheim) according to the manufacturer's conditions. One-half of the digestion reaction was electrophoresed on a 2% agarose gel according to standard procedures (Sambrook et al. 1989), and restriction fragments were detected with fluorescence of ethidium bromide stained bands under ultraviolet illumination.

Phylogenetic analysis.—To assign haplotypes to the major genetic lineages found in breeding populations around the world (Wenink et al. 1994), a neighbor-joining tree (Saitou and Nei 1987) of all haplotypes was computed from a matrix of corrected pairwise distances calculated under maximum likelihood in PHYLIP (Felsenstein 1991). Transversions, insertions, and deletions were weighted 4.75 times the transitions based on the empirical frequencies of these types of mutations. We included insertions and deletions because they also are relatively uncommon and thus are potentially phylogenetically informative. Sequences of the Purple Sandpiper (*Calidris maritima*) were used to root the tree.

Morphometrics.—To examine the association of phenotypic characters with mtDNA haplotypes of nonbreeding Dunlins, we recorded phenotypic characters from the birds we sequenced. Bill length (exposed culmen) was measured to the nearest 0.1 mm, and wing length to the nearest mm. Body mass was determined to the nearest gram within 2 h of capture. Adult buff phenotype of migratory Dunlins was assessed according to the criteria of Gromadzka (1986). Sex was distinguished where possible on the basis of presence (male) or absence (female) of a white neck collar (Ferns and Green 1979). Regional comparisons of the association of bill size and mtDNA haplotype of individuals were made using haplotype data from Wenink et al. (1994). Accordingly, the Baltic Region comprises birds from southern Sweden, Germany, and Denmark; western Siberia comprises birds from the Yamal Peninsula; and central Siberia comprises birds from the Taymyr Peninsula and the Lena River delta. Ranges of bill sizes were taken from Greenwood (1986), whose corresponding locality numbers are as follows: Iceland (2), southern Norway (8), Baltic (6 and 9), northern Norway (10), western Siberia (12), and central Siberia (13).

RESULTS

Sequence polymorphism.—Fifty-two Dunlins from migratory routes and wintering grounds around the world (Fig. 1) were each analyzed for 608 bases of mtDNA control-region se-

TABLE 2. Variable sites in segment I (295 base pairs [bp]) and segment II (313 bp) of the mtDNA control region of 52 migratory or wintering Dunlins. Sites are numbered according to their position in the Dunlin control-region sequence (Wenink et al. 1994). Dots indicate identity with top sequence and dashes indicate gaps introduced for alignment. Haplotypes abbreviated as in Table 1 and their frequency indicated in brackets.

	Position no. in control region	
	I	II
	11111222222223333333333	6777777
	27999022555782222345667	0111244
Haplotype/specimens	23368103578969456744812 7	2029089
LINEAGE I		
1 CAF (3)	ACTCACGACCTGTATAACCTCGAG	CAATCGT
2 HON (2), KAM (1)	......A.....	.....
3 WAS (2), CAF (1)	......A.....G.....	.....
4 WAS (1)	GT....A.....	.G.....
LINEAGE II		
5 HON (1)	....G.A.T..A.G....T...GA	.G.....
6 HON (1)	..C.G.A.T..A.G....T...GA	.G.....
7 CAF (1)	G.....A.T..A.G.....GA	.G.....
8 WAS (1)	G...G.A.T..A.G.....A	.G.....
LINEAGE III		
9 GUL (3), GDA (2)	....G.A.....C.C..T.....	.G..T..
10 WAD (2), GDA (1)	....G.....C.C..T.....	.G..T..
11 KRY (1)	....G.A.....C.C..T.C....	.G..T..
12 GUL (2), GDA (1), WAD (1)	....G..G....C...TT.....	.G..T..
LINEAGE IV		
13 WAD (4), GDA (3), KRY (3), GUL (3)	.T....A..AC..G.....CTA.A	.G..T..
14 WAD (1)	.T..G.A..AC..G.....CTA.A	.G..T..
15 KRY (1)	.T....A..AC.....CTA.A	.G..T..
16 WAD (1)	.T....A..AC..G..G..CTA.A	.G..T..
17 WAD (1)	......A..AC..G....TCTA.A	.G..T..
18 WAD (1)	.T....A..AC.....T.CTA.A	.G..T..
19 GDA (1)	.T....A..AC..G.....CTA.A	.G..TAG
20 WAD (1)	.T.TGTA..AC..G.....CTA.A	.G..T..
21 WAD (1)	.T.T.TA..AC..G.G...CTA.A	.G..T..
22 WAD (2)	.T....A..AC..G.....CTA.A	.G-CT..
23 WAD (1)	.T....A..AC..G.....CTA.A	AG-CT..
24 KRY (1)	.T..G.A..AC..G.....CTA.A	.G-CT..

quence. Sequence comparison revealed that control-region segment I differs at 24 nucleotide positions and that segment II differs at 7 nucleotide positions in these birds. These polymorphic sites together define 24 haplotypes (Table 2).

Phylogeny of haplotypes.—A neighbor-joining tree based on haplotypes found in both nonbreeding and breeding birds grouped the 24 haplotypes of the migrants into four different phylogenetic clusters (Fig. 2). The haplotypes found in breeding Dunlins (Wenink et al. 1996) were included in the tree analysis to help allocate individuals to the major mtDNA lineages found around the world. The tree demonstrates that the 24 haplotypes found in the nonbreeding birds belong to four of the five major mtDNA lineages present on the breeding grounds. No new major lineages were detected in this sample of migratory and wintering Dunlins and, furthermore, their haplotypes are either identical with or very similar to those from breeding Dunlins. The mtDNA lineage present in birds breeding in Canada (Wenink et al. 1993) is not represented in this study.

Geographic spread and mixing of mtDNA lineages.—Each stopover and wintering locale assayed (locales 1–8) revealed a mixture of two major mtDNA lineages, with the exception of locale 6, which contained a single migrant. Locales 1 to 4 (Wadden Sea, Gdansk, Krym and Persian Gulf) contained haplotypes belonging

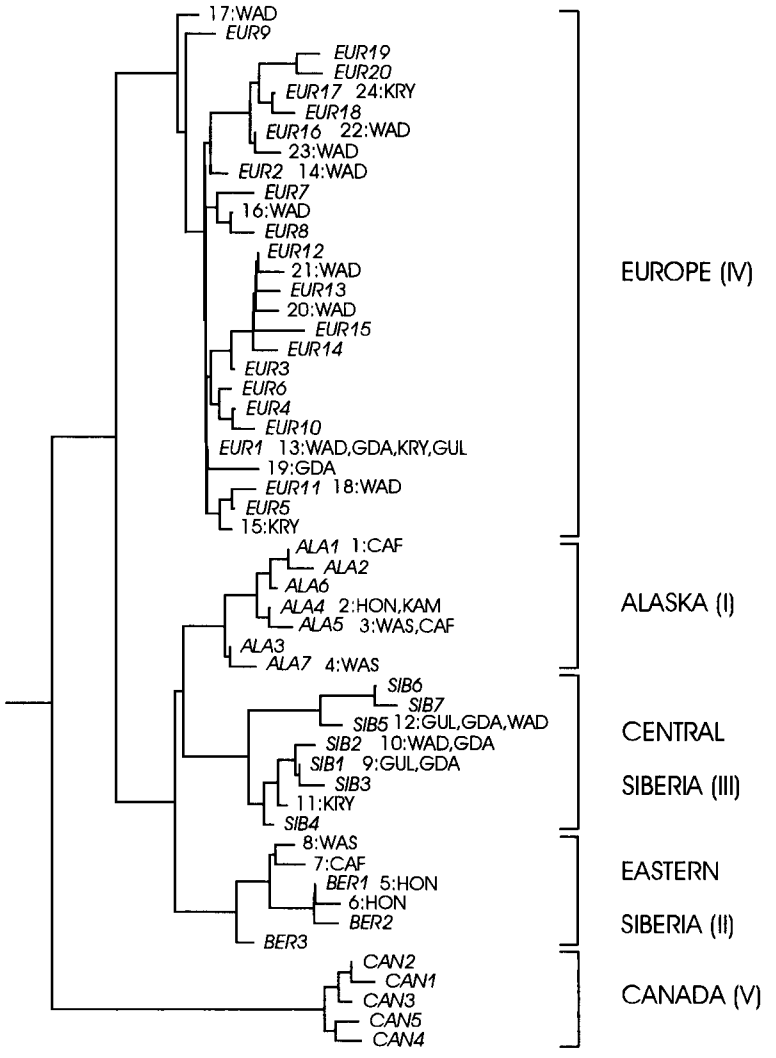

FIG. 2. Neighbor-joining tree depicting relationships between haplotypes of migratory and wintering Dunlins, and previously identified haplotypes of breeding Dunlins (italics) based on Wenink et al. (1996). Brackets and labels to right identify phylogeographic groups of breeding Dunlins.

to lineages III and IV, whereas locales 5, 7, and 8 (Hong Kong, Washington and California) provided combinations of haplotypes from lineages I and II (Table 2).

The frequency of each lineage at locales needs to be interpreted with caution because of the small numbers of birds analyzed. Additionally, a number of migratory birds from the Wadden Sea and Gdansk were selected for mtDNA analysis based on a phenotype presumed to indicate a Siberian breeding origin, or on the basis of banding recoveries (see below). Despite this selection, only 3 of 16 Wadden Sea birds and 4 of 8 birds from Gdansk possessed a lineage III (central Siberia) haplotype. Of the birds sampled from the Krym and the Persian Gulf, 1 of 6 and 5 of 8 birds belonged to this lineage, respectively.

The 25 Dunlins that belong to lineage IV (Europe) collectively possess12 haplotypes. Seven of these European haplotypes were not observed among breeding Dunlins (Fig. 2) and occur only in individual birds. Haplotype 13 is present at all four locales where Dunlins of lineage IV were found (Table 2). This haplotype occurs at a similarly high frequency (52%) among

TABLE 3. Distribution by locale of lineage I haplotypes in Dunlin.

Haplotype[c]	Alaska breeding locale[a]			Migration or wintering locale[b]			
	West	South	North	HON	KAM	WAS	CAF
ALA1	5	5	4	—	—	—	3
ALA2	—	1	—	—	—	—	—
ALA3	—	—	2	—	—	—	—
ALA4	1	1	9	2	1	—	—
ALA5	—	1	—	—	—	2	1
ALA6	1	—	—	—	—	—	—
ALA7	—	—	—	—	—	1	—

[a] West = Chevak; South = Cordova; North = Barrow.

[b] Abbreviations same as in Table 1.

[c] Haplotypes identified as in Figure 2, consistent with designations in Wenink et al. (1993). Haplotypes 1 to 4 in Table 2 are synonymous with ALA1, ALA4, ALA5, and ALA7, respectively.

breeding Dunlins (referred to as EUR1 by Wenink et al. 1996). Another lineage IV haplotype (EUR12) that was relatively abundant (13%) in breeding Dunlins is absent from the current sample of nonbreeders. This haplotype was largely confined to birds breeding in Iceland (Wenink et al. 1996). Two birds from the Wadden Sea reveal haplotypes (20 and 21) closely related to this Icelandic haplotype (Fig. 2). Both haplotypes have two *T* substitutions in the control region I segment (at positions 196 and 201 in Table 2) in common with the Icelandic sequence.

Too few representatives of the other three lineages were present in the nonbreeding birds to justify a frequency comparison with birds from the breeding grounds (lineage I, $n = 10$; lineage II, $n = 4$; lineage III, $n = 13$). However, it was possible to compare the presence or absence of individual haplotypes in nonbreeding and breeding birds. Seven lineage I haplotypes have thus far been found among 30 Alaskan breeding and 10 migrant or wintering Dunlins (Fig. 2, Table 3). Haplotype ALA1 occurred commonly at all Alaskan breeding locales, and was found in three Dunlins wintering in California. Haplotype ALA4 was most numerous among northern Alaskan breeding birds (present in 9 of 15 birds) and was found in only two Dunlins breeding elsewhere in Alaska. ALA4 was not present among the seven Dunlins belonging to lineage I wintering along the west coast of North America, but instead occurred in all three Dunlins of this lineage sampled on migration along the east coast of Asia (Table 3).

Lineage II haplotypes previously were found in Dunlins breeding in far eastern Siberia, but not in Alaska (Wenink et al. 1996). Haplotypes of this lineage were detected in Dunlins sampled in Hong Kong, but also in two Dunlins wintering along the west coast of North America (Table 2).

Allocation of migratory and wintering Dunlins of lineages III (central Siberia) and IV (Europe) to their breeding populations cannot be made with certainty because of limited exchange of individuals between these phylogeographic groups on the breeding grounds. Examination of additional phenotypic characters may aid in revealing the breeding origins of Dunlins belonging to these lineages in composite winter flocks.

Other population markers and mtDNA.—Average bill lengths and standard deviations for six European and Siberian populations are plotted in Figure 3. For both sexes there is a stepwise increase in average bill length from the populations in Iceland, southern Norway, and the Baltic to the populations in western and central Siberia, although size ranges of these populations clearly overlap. The breeding population in northern Norway occupies an intermediate position. Large bill length for both sexes is indicative, but not diagnostic, of a Siberian breeding origin. Bill lengths of individual breeding Dunlins for which the mtDNA sequence has been determined (Wenink et al. 1996) are plotted above the population size ranges in Figure 3. Bill lengths of some individuals are markedly divergent from the population mean and overlap into the range of populations of different mean size. Consequently, bill length is significantly but weakly correlated with the mtDNA of individual birds (males: $r = 0.36$, $n = 52$, $P < 0.01$; females: $r = 0.39$, $n = 47$, $P < 0.01$). Four male Dunlins in the southern and northern

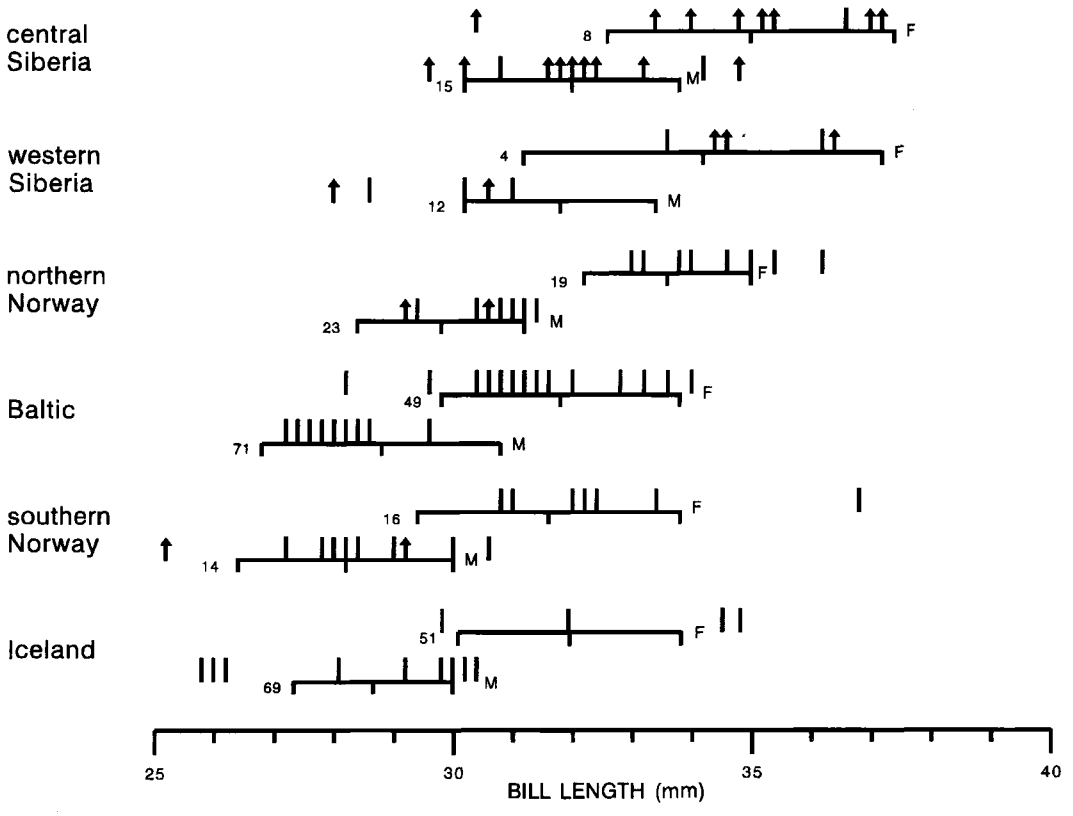

FIG. 3. Relationship between bill size and mtDNA haplotype in 99 breeding Dunlins from six regions. Males (M) and females (F) plotted separately. Vertical tick marks and horizontal lines depict bill length ($\bar{x} \pm$ SD) for each region, with sample sizes to the left. Bill lengths of Dunlins with a lineage IV haplotype are marked by vertical lines, and Dunlins with a lineage III haplotype are marked by vertical arrows.

Norway populations possess a lineage III (central Siberia) haplotype, but are indistinguishable in bill size from the other Dunlins in their breeding population. This also is true for the three central Siberian Dunlins with an immigrant lineage IV (Europe) haplotype. The western Siberia region (Yamal Peninsula) previously has been revealed as a zone of overlap between phylogeographic groups III and IV (Wenink et al. 1996). Because of potential interbreeding between birds of these two populations, unisexually inherited mtDNA cannot unequivocally assign breeding birds from this zone to a phylogeographic group.

Table 4 presents mtDNA haplotype, morphometric measures, date of capture, and banding recoveries for 36 migratory and wintering Dunlins from locales 1 to 4 (thus representing lineages III and IV). Six individuals had very long bills: 012 (37.7 mm), 235 (38.6 mm), 242 (39.8 mm) from the Wadden Sea, GD06 (37.4 mm) from Gdansk, IC12 (37.5 mm) from Krym, and EH17 (38.5 mm) from the Persian Gulf. Only two of these (012 and EH17) possessed a haplotype belonging to the central Siberian lineage.

Whereas Dunlins breeding in northern Europe attain peak numbers at the Dutch Wadden Sea in April, those breeding in Siberia are thought to pass through predominantly in May. The second group of migrants attains a higher percentage of body fat during spring migration, presumably in preparation for longer flights to the arctic (Goede et al. 1990). Four heavy individuals were caught on spring migration in the German Wadden Sea in May: 012 (90 g), 140 (70 g), 143 (66 g), and GD12 (75 g; bird recaptured). Three of these birds had a lineage III haplotype (Table 4).

Dunlins breeding east to the Yamal Peninsula

TABLE 4. Genetic and phenotypic data for 36 nonbreeding Dunlins from western Palearctic.

Individual	Haplotype/ lineage	Bill (mm)	Wing (mm)	Sex	Mass (g)	Capture/ Recovery	Remarks[a]
012 Wad	10/III	37.7	127	F	90	26 May	Very heavy
042 Wad	20/IV	33.6	—	U	51	13 Aug	England (4 Mar)
044 Wad	14/IV	34.1	118	U	52	13 Aug	England (8 Apr)
048 Wad	22/IV	31.2	118	—	45	13 Aug	
049 Wad	23/ IV	35.1	—	—	58	13 Aug	
051 Wad	13/IV	34.4	127	F	53	13 Aug	
054 Wad	10/III	32.1	121	—	50	13 Aug	England (24 Feb)
140 Wad	13/IV	34.9	119	F	70	14 Jun	Very dark
143 Wad	12/III	35.7	126	F	66	14 Jun	Netherlands (3 Nov)
235 Wad	13/IV	38.6	118	—	60	26 Jul	Very large bill
240 Wad	13/IV	29.3	114	—	42	26 Jul	Early arriving juvenile
242 Wad	17/IV	39.8	121	F	60	29 Jul	Very large bill
248 Wad	22/IV	30.0	116	F	50	9 Aug	Very fresh primaries
262 Wad	18/IV	30.6	116	F	51	23 Jul	
GD06 Gda	19/IV	37.4	123	—	47	15 Jul	England
GD07 Gda	13/IV	33.6	125	—	51	15 Jul	Finland
GD08 Gda	13/IV	35.4	120	—	45	16 Jul	England
GD12 Gda	9/III	36.3	121	M	50	18 Jul	Germany (18 May: 75 g)
GD16 Gda	10/III	35.2	118	—	48	24 Jul	Krym (31 May)
GD17 Gda	13/IV	31.3	115	—	50	24 Jul	Finland
GD21 Gda	12/III	31.6	—	—	44	27 Jul	Adult buff, Norway
GD22 Gda	9/III	31.3	121	—	41	27 Jul	Adult buff
IC04 Kry	13/IV	31.5	119	—	44	13 Aug	Adult buff?
IC07 Kry	11/III	35.3	122	—	49	13 Aug	Poland (20 Jul)
IC08 Kry	13/IV	31.5	121	—	45	13 Aug	Poland (28 Jul)
IC09 Kry	13/IV	33.0	—	—	45	9 Sep	Poland (15 Aug)
IC11 Kry	24/IV	35.4	123	—	50	9 Sep	Adult buff?
IC12 Kry	15/IV	37.5	121	—	48	9 Sep	
PS02 Gul	13/IV	29.6	114	—	37	21 Nov	Juvenile
PS06 Gul	12/III	31.2	116	—	44	21 Nov	Juvenile
PS07 Gul	12/III	36.0	127	—	54	21 Nov	Juvenile
PS10 Gul	13/IV	32.9	120	—	49	21 Nov	Juvenile
EH01 Gul	9/III	35.1	125	—	45	23 Sep	Juvenile
EH03 Gul	13/IV	31.7	115	—	41	23 Sep	
EH05 Gul	9/III	30.2	118	—	45	24 Sep	Juvenile
EH17 Gul	9/III	38.5	121	—	53	27 Sep	

[a] Including information on original encounters with birds.

in Siberia start molting on the breeding grounds and, presumably, can be distinguished from more westerly breeding birds on the basis of their new median wing coverts (adult buff) during fall migration (Gromadzka, 1989). Two of eight fall migrants from Gdansk that were inspected for this feature had the adult buff phenotype and also had a central Siberian haplotype (GD21, GD22). The other two Dunlins from Gdansk belonging to lineage III (GD12 and GD16), however, were not identified by adult buff coverts (Table 4).

The most direct way to determine Dunlin breeding origins is to recapture individuals banded on the breeding grounds. This strategy, however, is frustrated by the low number of recoveries and the large efforts involved in banding birds on the extensive and remote arctic breeding grounds. Five banded Dunlins possessed a lineage III haplotype (054, GD12, GD16, GD21, IC07), but none of these birds was banded on the breeding grounds (Table 4).

Lineage assignment in Europe using restriction analysis of mtDNA.—Based on the available sequence information for Dunlins, it is possible to design an assay that discriminates between birds that mix in their migratory routes and winter distributions in Europe. Both control-region segments were scanned for the presence or absence of a restriction site as a result of one of the diagnostic DNA substitutions between lineages III and IV. Only control-region segment I differs at four positions (257, 258, 358, and 361 in Table 2), and it does so consistently

for all individuals belonging to these lineages, including 29 central Siberian and 85 European breeding Dunlins (Wenink et al. 1994). The substitution at position 358 in the lineage III control-region sequences creates an *Alu I* restriction site that is absent from the lineage IV sequences (Fig. 4A). A *Taq I* site at position 361 is of no use because of the presence of another *Taq I* site very nearby. Digestion of the control region I DNA segment of nine Dunlins from the Wadden Sea with *Alu I* revealed that three birds had a central Siberian haplotype. The PCR product remained uncut in six birds, indicating that they were European haplotypes lacking the mutation that created this cut site (Fig. 4B). Each digestion pattern is in agreement with the assignment using DNA sequence information.

DISCUSSION

Mixing of populations during the nonbreeding season.—Mixing of mtDNA lineages was observed in migratory and wintering populations of Dunlins from widely distributed locales around the world. This finding is in contrast with the subdivided population structure over the Dunlin's circumpolar breeding range (Wenink et al. 1993, 1996). The four mtDNA lineages detected in nonbreeding birds are identical to those found previously among breeding Dunlins. Fifteen of the 24 haplotypes were observed for the first time. All but one were restricted to individual birds, suggesting that the most frequent haplotypes per lineage have been recovered in our previous extensive assay of breeding populations. Because mtDNA lineages in breeding and nonbreeding birds are identical, it is possible to assay Dunlins captured on migration or at wintering sites to determine their breeding provenance (see below).

Mixing of birds from two phylogeographic groups occurred in the Pacific area (lineages I and II) and in the western Palearctic (lineages III and IV). In both cases, the mixing of lineages involved geographically contiguous breeding populations. No admixture of lineages II and III was observed, although such a situation could exist in central to eastern Asia, and may not have been detected at locales 5 and 6 because of the small sample sizes we analyzed. No other combinations of lineages are expected, assuming that all lineages in Dunlins have been discovered and that the fifth lineage within eastern North America is geographically isolated (Maclean and Holmes 1971). The global distribution pattern of lineages indicates a substantial overlap of Dunlin populations on southward migration and in their winter range. This same conclusion was reached based on band recoveries (Greenwood 1984).

Pacific migration routes.—Dunlins belonging to lineages I and II that breed on either side of the Bering Strait appear to migrate along both sides of the Pacific Ocean. Two of nine Dunlins from the west coast of North America possessed a lineage II haplotype that so far has been found only in birds breeding in far eastern Siberia. Equally as important, no lineage II haplotypes have been observed in 30 breeding Dunlins from three locales in Alaska. The presence of migrant Siberian Dunlins in western North America has until now gone unnoticed, presumably because no extensive banding program has been undertaken on the eastern Siberian breeding grounds. Three of five migrant Dunlins assayed from the eastern coast of Asia had an Alaskan lineage I haplotype. Because only four breeding birds from far eastern Siberia have been analyzed to date, the possibility that Dunlins with a lineage I haplotype breed on both sides of the Bering Strait cannot be excluded. If there is complete phylogeographic subdivision of lineages I and II, then a northern Alaskan breeding origin is indicated for the Asian migrants because their haplotype occurs at high frequency only near Barrow in northern Alaska. A separate migration route along the eastern coast of Asia for Dunlins from northern Alaska also has been proposed on the basis of several band recoveries (Norton 1971). However, considerably

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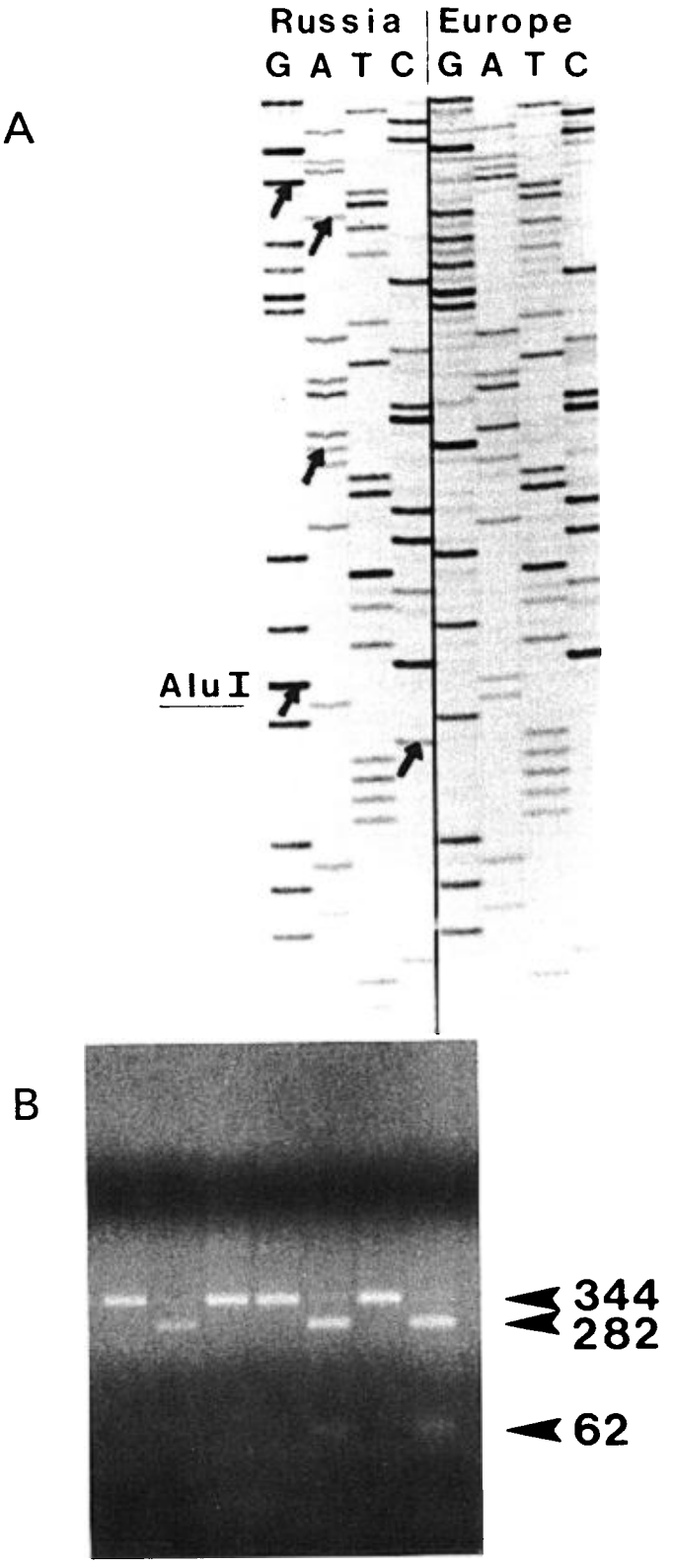
FIG. 4. (A) Part of control region I sequence of a Dunlin with lineage III haplotype (Russia), and a Dunlin with lineage IV haplotype (Europe). Base substitutions in this part of sequence indicated by arrows in Russian sequence. The *Alu I* restriction site (AGCT) in the Russian sequence is also indicated. (B) *Alu I* restriction digestion pattern of control region I amplification product for nine Dunlins from the German Wadden Sea. Sizes of restriction fragments (including primers) given in base pairs to right. The 62-bp fragment is faint due to its small size.


A
Russia
Europe
G A T C
G A T C
AluI
B
344
282
62

larger sample sizes will have to be analyzed to check this preliminary result.

Western Palearctic population composition.—The predominance of lineage III haplotypes in the Persian Gulf, although based on a small sample of eight birds, supports the notion that this region has a large influx of Dunlins from the central Siberian breeding population (Vieillard 1972). Overall, however, the allocation of Dunlin populations to specific breeding grounds in the western Palearctic is not straightforward. This uncertainty is caused by an overlap of breeding ranges and the exchange of a small proportion of haplotypes between breeding groups III and IV. Excluding individuals from the zone of intergradation in western Siberia, 3 of 22 Dunlins from the central Siberian group (III) possessed an immigrant lineage IV haplotype, and 4 of 81 Dunlins from the European group (IV) had a lineage III haplotype (Wenink et al. 1996). All immigrant lineage III haplotypes were found in Norway (n = 33), but not in Iceland (n = 17) or the Baltic Region (n = 27), suggesting a restricted northern European migration route for their dispersal (Wenink et al. 1996). Some of the birds with a lineage III haplotype present in the European Wadden Sea or the eastern Baltic (Gdansk) may therefore derive from the northern European breeding grounds, rather than being true representatives of the central Siberian breeding population. This fraction will likely be larger in the Wadden Sea than near Gdansk because the Wadden Sea is a southward extension of the migratory route along the western Norwegian coast (Leslie and Lessells 1978), whereas many birds passing Gdansk are thought to migrate overland to the Mediterranean and Black Sea (Gromadzka 1989).

Genetic-phenotypic correlations.—The presence of Siberian Dunlins at European locales has been suggested on the basis of several characteristics. Siberian birds are predicted to have large bills and wings (Engelmoer et al. 1987), delayed migration in combination with increased body mass in spring (Goede et al. 1990), and a specific molting pattern in fall (Gromadzka 1989). Additionally, birds bearing European bands have been recovered on Siberian breeding grounds east of the Ural Mountains (ca. 60°E; Gromadzka 1985, 1989). Only molting behavior shows a geographic specificity that corresponds roughly with the phylogeographic subdivision between breeding groups III and IV. In both cases, the zone of overlap covers the Yamal Peninsula in west-central Siberia (ca. 70°E; Wenink et al. 1996). Bill and wing size reveal no discrete differences across this range (Greenwood 1986; Fig. 3 for bill length). Observations at staging posts during spring migration are hard to relate to breeding range, although Goede et al. (1990) tentatively calculated the maximum flight distance of western European Dunlins to be near the western end of the Taymyr Peninsula in central Siberia (ca. 80°E), based on the rate of increase in body mass.

Two of six migratory and wintering Dunlins with very long bills thought to be indicative of a Siberian breeding origin possessed lineage III haplotypes. The remaining four birds had a lineage IV haplotype and could have originated from breeding grounds in western Siberia (e.g. Yamal Peninsula), but they were highly unlikely to have come from breeding grounds much farther to the east because of the prevalence of lineage III haplotypes there (Taymyr Peninsula). Use of bill length for the determination of breeding origin of individual Dunlins *a priori* is complicated by the overlap of population size ranges. Most overlap results from sexual dimorphism (Fig. 3), and the method would, therefore, profit considerably from reliable sex determination of migratory and wintering birds. A general solution to this problem could be the amplification of female-specific sequences that are located on the avian *W* chromosome (Griffiths and Tiwari 1993). Four sexed Norwegian breeding Dunlins with short bills had an immigrant lineage III haplotype (Fig. 3), presumably reflecting paternal contribution(s) to a nuclear-encoded character (bill size).

Three of four Dunlins with heavy body masses in the German Wadden Sea in May were found to have a lineage III haplotype. Two of eight Dunlins passing through the eastern Baltic on fall migration were diagnosed as having adult buff phenotypes; they also belonged to this mtDNA lineage. Dunlins from the Krym and the Persian Gulf were not considered for the above two criteria, as the adult buff character is difficult to recognize in wintering Dunlins because of increased feather wear, and because a differential fattening regime is not present in Dunlins at wintering sites. Overall, a Siberian breeding origin was indicated for five of the seven migrants from the Wadden Sea and Gdansk possessing a lineage III haplotype. This correlation between mtDNA lineage and the phenotypic criteria suggests that only a small

fraction of the lineage III haplotypes present in northern European migrants originates on the Norwegian breeding grounds. An extended mtDNA analysis of samples of European migrants from both spring and fall is needed to evaluate comprehensively the correspondence between these phenotypic and genetic measures, and their utility for the diagnosis of birds with a Siberian breeding origin. The *Alu I* restriction assay will suffice for this particular goal.

These initial results encourage further evaluation of the genetic composition of Dunlin populations outside of their breeding range. Using the sequence information from the breeding range as a source of reference, mtDNA analysis of migratory and wintering Dunlins can elaborate on the global genetic architecture of the species and also can reveal the broad migratory routes that Dunlins use between northern and southern ranges. Finally, mapping of the wintering sites of particular breeding populations with mtDNA markers seems to be feasible, and should prove to be an invaluable tool in the conservation biology of Dunlins.

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