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Breeding origin and migration pattern of dunlin (*Calidris alpina*) revealed by mitochondrial DNA analysis

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Abstract

The large-scale migration of birds has been studied extensively by recoveries of ringed birds. However, there is very little ringing data from the arctic breeding grounds of waders. Here, the migration pattern of the dunlin, *Calidris alpina*, is studied with population genetic markers, using haplotype frequencies to estimate the breeding origin of migrating and wintering populations. Polymerase chain reaction (PCR) and restriction analysis of DNA from the mitochondrial control region was used to study the breeding origins of morphologically similar winter populations in the western Palaearctic, and to describe the population structure of the dunlin during winter. Also migrating dunlin from various stopover sites in Europe, Africa and Asia, were analysed with respect to their mitochondrial DNA (mtDNA) haplotypes. The genetic markers clearly show that the dunlin has a parallel migration system, with populations breeding in the western Palaearctic wintering mainly in the western part of the wintering range, and dunlin populations breeding further east wintering further east. The results also show that the distance between breeding and wintering area increases eastwards in this region.

Keywords: *Calidris alpina*, dunlin, migration, mtDNA, PCR, population genetics

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Introduction

Genetic markers are often used to reveal the phylogenetic origin of species or populations (e.g. Sibley & Ahlquist 1990). They have also been used to investigate the historic origin of populations colonizing new geographical areas by dispersal (e.g. Cann *et al*. 1987; Avise & Nelson 1989; Quinn *et al*. 1991). In this study, genetic markers were applied in a different way, involving studies on a much shorter time scale. They were used to trace the geographical origin of migrating birds. The distribution of birds with different haplotypes in the breeding areas was used to estimate the breeding origin of migrating and wintering populations, making it possible to recognize different populations throughout their annual cycle, following their spectacular annual migration between breeding and wintering areas.

For migrating birds, following the same population all year around can be quite a challenge, as they may be found in widely separated geographical regions during different parts of the year. The dunlin, *Calidris alpina*, is a long-distance migrant with a circumpolar breeding distribution. To estimate the origin of winter populations of dunlin, several morphological characters have been used (e.g. bill, tarsus, wing and total length, as well as plumage coloration). These are characters that vary between subspecies (Glutz von Blotzheim *et al*. 1975; Prater *et al*. 1977; Cramp & Simmons 1983; Greenwood 1986; Engelmoer & Roselaar 1998). However, all characters show substantial overlap between subspecies. In combination with the great morphological variation among birds at each breeding site, this has made it very difficult to identify the breeding origin of migratory and wintering dunlin. Sexual dimorphism further complicates the identification of populations, as the sex of a dunlin is often difficult to identify (Pienkowski & Dick 1975; Prater *et al*. 1977; Cramp & Simmons 1983).

In addition, ringing data from the arctic breeding grounds are very scarce and there are, therefore, extremely few ringing recoveries linking breeding populations to wintering areas (Greenwood 1984). In this study, I tried using genetic markers to solve the problem of identifying migratory birds throughout the year. This requires good knowledge about the genetic differences between populations, as well as a limited gene flow between them.

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Haig *et al.* (1997) used random amplified polymorphic DNA (RAPD) and found differences in band pattern among three Nearctic populations of dunlin. Using a more specific method, a larger sample was analysed by Wenink *et al.* (1993, 1996), who sequenced DNA from the mitochondrial control region and found five major mitochondrial DNA (mtDNA) lineages in dunlin from all over the world. These different phylogenetic groups of dunlin can now be identified easily using restriction analysis (Wenink & Baker 1996; Wennerberg *et al.* 1999), and have also been identified in samples from migrating dunlin (Wenink & Baker 1996; Tiedemann 1999). Further data have accumulated since the study of Wenink *et al.* (1996), showing the mitochondrial haplotype distribution of the dunlin on the Palaearctic breeding grounds in some geographical detail (Wennerberg *et al.* 1999; this study). The frequency of mtDNA haplotypes varies according to a consistent geographical pattern within the breeding range. This makes it possible to use genetic markers to estimate the possible breeding origins of dunlin on stopover sites during migration or at winter sites.

In this paper, a map is presented with the distribution of each mtDNA haplotype of dunlin in the breeding grounds, in which all the available results from the breeding areas are summarized and new data are included (Wenink *et al.* 1993, 1996; Wennerberg *et al.* 1999).

Mitochondrial DNA haplotypes were used to study the migration pattern of dunlin in the western Palaearctic, analysing the haplotype frequencies of 752 migrating and wintering dunlin at 22 localities. The genetic composition of dunlin at different stopover and wintering sites was investigated and used to map the distribution of dunlin populations in winter, as well as to estimate the possible breeding origins and migration distances of winter populations. In addition, the results of the mtDNA analysis were used to discuss the migration routes and general migration patterns of the dunlin.

Materials and methods

A total of 752 migrating and wintering dunlin was captured at 22 localities in Europe, Africa and Asia (Table 1). The results included 26 Moroccan and 102 Swedish dunlin which were also included in two other studies (L. Wennerberg & J.L. Arroyo, unpublished manuscript; L. Wennerberg, N.M.A. Holmgren, P.E. Jönsson & J. Pettersson, unpublished manuscript).

Blood samples of 20–50 μL were taken from all birds. Samples were stored in 95% alcohol or in SET-buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH = 8.0). DNA was extracted using the following method: ≈ 2 μL of blood was incubated with 250 μL 5% Chelex (Bio-Rad) and 3 μL Proteinase K (20 mg/mL) at 55 °C for 5 h. The sample was then mixed, boiled for 8 min, mixed again and centrifuged for 2 min, after which the top 100 μL was removed and used for further analyses (below).

Two parts of the control region of the mtDNA (region I and II) were amplified by polymerase chain reaction (PCR), using the primers from Wenink *et al.* (1993). Primers L98 and H410 were used for region I and L438 and H772 for region II. PCR included 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s. The 25-μL reaction mix contained 1.0 μL DNA, 2.5 μL of each primer (10 μM), 2.5 μL 10 × PCR buffer II (Perkin–Elmer), 2.5 μL dNTP (1.25 mM of each nucleotide), 1.0 μL $MgCl_2$ (25 mM), 12.9 μL dH_2O and 0.1 μL *Taq* DNA polymerase (5 units/μL).

Thereafter, the PCR products were cut using specific restriction enzymes (Boehringer-Mannheim). The restriction enzymes were chosen to cut the DNA at positions which separate the four DNA lineages that occur on the Palaearctic breeding grounds (Wenink *et al.* 1996; Wennerberg *et al.* 1999). Samples of control region I from all individuals were incubated with the enzyme *Alu*I. This separates the European haplotype (E) from the Siberian (S), Beringian (B) and Alaskan (A) haplotypes. For all individuals that did not have the European haplotype, region I was incubated with *Rsa*I. This separates the Beringian haplotype from the others (E, S and A). Finally, the Siberian and Alaskan haplotypes were separated by incubating region II with *Dde*I. In all cases, the DNA fragments were separated in a 2% agarose gel containing ethidium bromide and visualized under UV-light. The length of each band was compared with reference bands of all haplotypes, as well as with a size marker (1 kb DNA ladder, Life Technologies). For further details on the method see Wennerberg *et al.* (1999).

The results from breeding dunlin are based partly on previously published work by Wenink *et al.* (1996) and Wennerberg *et al.* (1999), but also include 96 new samples, adding further detail to the distribution of the mtDNA haplotypes on the breeding areas. These additional samples were analysed in the same way as the samples of migrating and wintering birds (above).

The breeding origins of migrating and wintering populations were estimated by comparing their mtDNA haplotypes with those of breeding populations. Their haplotype compositions were related to a logistic regression of haplotype frequency and breeding longitude, including breeding populations in northern Scandinavia and Russia as far east as Lopatka Peninsula (149° E). This method is based on the assumption that the populations are structured according to isolation-by-distance, which was verified by analysis with GENEPOP 3.1b ($P < 0.001$, Raymond & Rousset 1995).

The haplotype compositions at the stopover and wintering sites were compared using Pearson's χ^2 test. If the sample sizes were too low for this test (according to SYSTAT 9, SPSS Inc.), Fischer's χ^2 test was used instead. The significance levels were adjusted by sequential Bonferroni technique (Rice 1989).

Table 1 Sampling locations, dates and mitochondrial DNA haplotypes of migrating and wintering dunlin

Location	*N*	Season	Eur	Sib	Dates
Bahrain (Dumistan)	26	autumn	7	19	23–30 September 1992
Germany (Norderheverkoog, Tetenbüllspieker)	15	spring	13	2	14–28 May 1991 29 March 1993
Germany (Westerhever, Dieksanderkoog-Süd)	13	autumn	7	6	22 July–4 September 1991 29 July–12 September 1992
Italy (Venice, Sardinia)	93	autumn	56	37	3 August–6 October 1997
Italy (Venice)	55	winter	26	29	11 December 1996 6 February 1997 1 November–27 December 1997
Italy (Venice)	69	spring	36	33	1 May 1992 4–5 May 1997 24 April 1998
Kazakhstan (Lake Tengiz)	8	autumn	4	4	22 August–23 October 1999
Morocco (Sidi Moussa)	26	spring	26	0	15–23 April 2000
Norway (Lista, Tromsø)	33	autumn	25	8	12 August–18 September 1992 2–3 September 1997
Poland (Gdansk Bay)	20	autumn	11	9	24–31 July 1996
Portugal (Mondego estuary)	53	autumn	53	0	5 August–3 October 1999
Portugal (Mondego estuary)	7	spring	7	0	23 April–22 May 1999
Russia (Yamal peninsula, Belyy Island)	16	autumn	6	10	20–23 August 1994
Spain (El Grove, Tarragona)	5	winter	2	3	29 November 1992–14 February 1993
Spain (Santona marsh, Tarragona)	14	autumn	11	3	3 August–29 September 1992
Sweden (Ottenby, Falsterbo)	193	autumn	130	63	18 July–5 October 1994 20 September–25 October 1995 13 September–23 October 1996
Sweden (Falsterbo)	13	winter	7	6	25 February 1994 27 February 1995 27 November–16 December 1995
Sweden (Falsterbo)	40	spring	33	7	12–29 April 1995 30 May 1996
Tunisia (Kneiss)	20	spring	7	13	14–28 March 1990 15–18 May 1990
Ukraine (Black Sea)	12	autumn	6	6	6 October 1991 7 October 1992 3 October 1997
Ukraine (Black Sea)	21	spring	8	13	26–27 May 1997
Totals	752		481	271	

Results

mtDNA haplotypes of breeding dunlin

Five mtDNA haplotypes (lineages) have been found in breeding populations of dunlin from all over the world (Wenink *et al*. 1996; Wennerberg *et al*. 1999; this study). The haplotype frequencies in the Palaearctic populations are shown in Fig. 1. In addition to the data shown in Fig. 1, mtDNA from four populations with low sample sizes have been analysed (N Sweden: 2E, Poland: 2E, Kanin peninsula: 1E, Vaigach Island: 2E), and one sample of mixed populations from the Taimyr peninsula (2E, 15S) was reported by Wenink *et al*. (1996).

In Iceland and in the Baltic region, only the European haplotype occurs (Fig. 1). These breeding populations belong to the subspecies *Calidris alpina schinzii* (Cramp & Simmons 1983). The subspecies *C. a. alpina* breeds in the arctic tundra of northern Scandinavia and northern Russia. The European haplotype predominates in the western part of this region, but, in contrast to the Baltic populations, these breeding areas also include dunlin of Siberian haplotype in low frequencies (Fig. 1). The proportion of the European haplotype decreases eastwards and the Siberian haplotype becomes increasingly more common in central Siberia. In northern Scandinavia and western and central Russia, as far east as the Lopatka Peninsula (149° E), the frequency of the European haplotype decreases with the

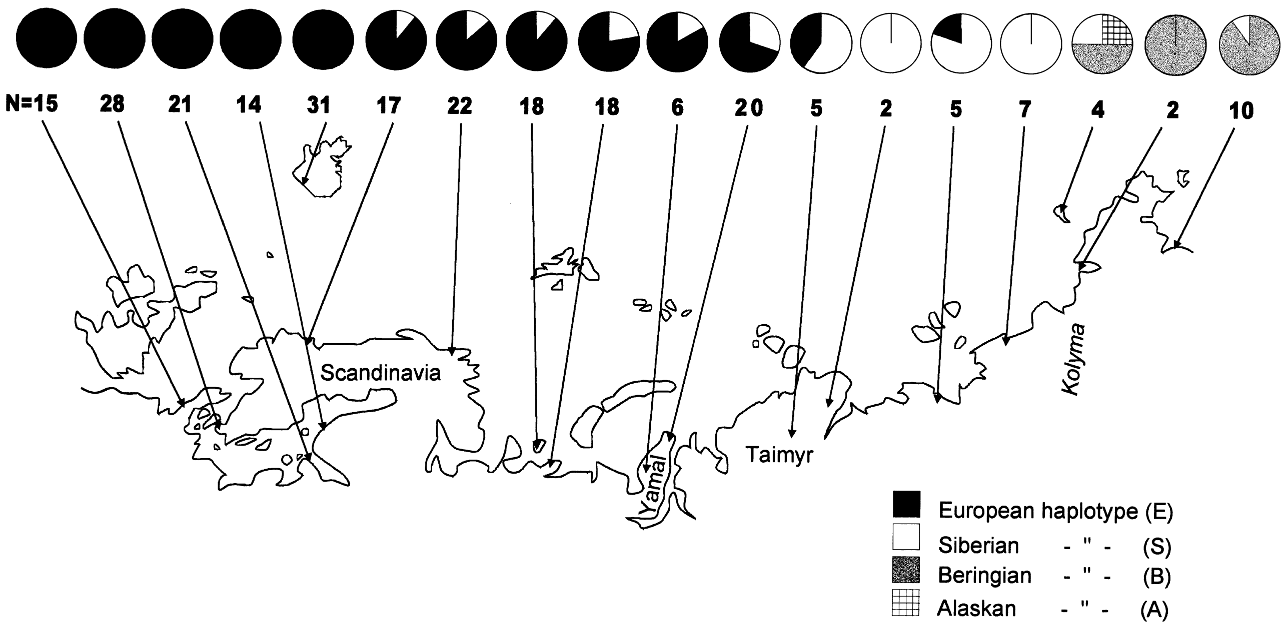

Fig. 1 Mitochondrial DNA haplotypes of breeding dunlin. The haplotype frequencies in each population are shown in circle diagrams, and the sample size (N) for each population is shown below the diagram. The figure includes samples from this study, as well as previously published data from Wenink *et al.* (1996) and Wennerberg *et al.* (1999).

longitude of the breeding site according to a logistic regression equation $y = 1 - 1/(1 + 58e^{-0.045x})$, where x is the longitude of the breeding site and y is the proportion of European haplotype ($\chi^2 = 0.06$, $R^2 = 0.93$, L. Wennerberg *et al.*, unpublished manuscript, Fig. 3). The central Siberian region, where the Siberian haplotype is predominant, is the breeding area of the disputed subspecies *C. a. centralis* (Buturlin 1932; Vaurie 1965; Dement'ev *et al.* 1969; Greenwood 1986). In eastern Siberia, the subspecies *C. a. sakhalina* is found (Cramp & Simmons 1983). Here, the Beringian haplotype predominates (Fig. 1). The Alaskan haplotype is found breeding mainly in Alaska, but has also been found among dunlin breeding on Wrangel Island in eastern Siberia (Fig. 1). The Canadian haplotype is found only in central Canada (Wenink *et al.* 1996).

Mitochondrial DNA haplotypes of migrating and wintering dunlin

Two mtDNA haplotypes of dunlin, the European and Siberian haplotypes, were found at the stopover sites and wintering localities in the western Palaearctic (Fig. 2, Table 1). In other words, no dunlin of Beringian or Alaskan haplotypes was found at any of these localities, including sites as far east as Bahrain and Kazakhstan. The haplotype frequencies differ significantly between sampling localities ($\chi^2 = 98.4$, d.f. = 12, $P < 0.001$). The frequency of birds with European haplotype varies between 27 and 100%. All the other birds have the Siberian haplotype.

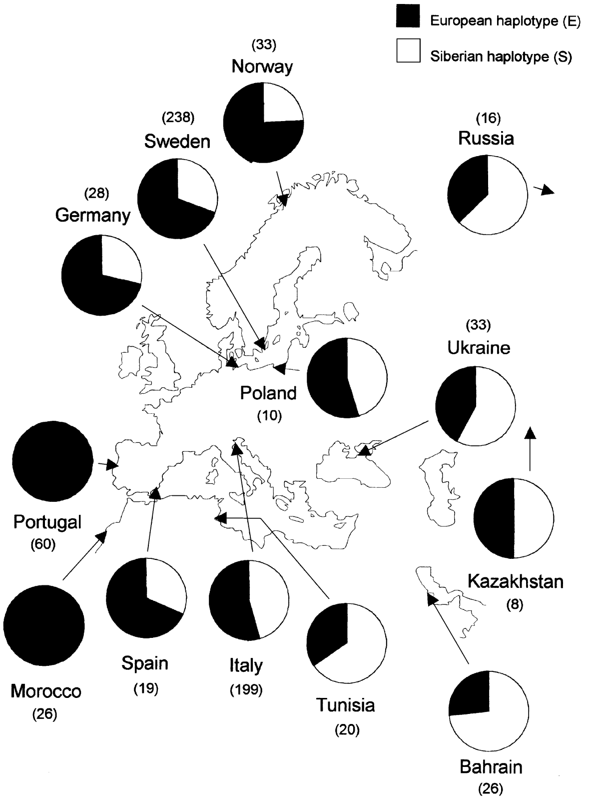

Fig. 2 Mitochondrial DNA haplotypes of migrating and wintering dunlin. The haplotype frequencies of each sample are shown in circle diagrams, where the European haplotype (E) is indicated in black and the Siberian haplotype (S) in white. The sample sizes (N) are shown in brackets. (See Table 1 for sampling locations and dates.)

Table 2 Chi-square tests comparing mtDNA haplotype frequencies of dunlin from different sampling localities. The significance levels are adjusted by sequential Bonferroni technique (Rice 1989) and indicated by asterisks *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Tendencies ($p < 0.1$) are indicated by n.s. (*)

	Bahrain	Germany	Italy	Kazakhstan	Morocco	Norway	Poland	Portugal	Russia	Spain	Sweden	Tunisia
Bahrain												
Germany	n.s.(*)											
Italy	n.s.	n.s.										
Kazakhstan	n.s.	n.s.	n.s.									
Morocco	***	n.s.	***	n.s.(*)								
Norway	*	n.s.	n.s.	n.s.	n.s.							
Poland	n.s.	n.s.	n.s.	n.s.	**	n.s.						
Portugal	***	**	***	**	n.s.	**	***					
Russia	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	***				
Spain	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.			
Sweden	**	n.s.	n.s.(*)	n.s.	*	n.s.	n.s.	***	n.s.	n.s.		
Tunisia	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	n.s.	n.s.	
Ukraine	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.

The highest proportion of the European haplotype is found in Portugal and Morocco, where all sampled individuals have the European haplotype. The haplotype frequencies at these two sites are significantly different from most other places where migrating or wintering dunlin have been sampled (Table 2). In Spain, the frequency of the European haplotype is 68%. In contrast to the Portuguese sample, which has only the European haplotype, the Spanish sample contains a relatively large proportion of birds with the Siberian haplotype. This is also the case for the central Mediterranean samples, Italy having 54% and Tunisia only 35% European haplotype (Table 2).

Three north European countries (Sweden, Germany and Norway) have similar haplotype frequencies with 69–76% European haplotype. The frequency of the European haplotype here is higher than in wintering areas further south-east (Table 1, Fig. 2). The sample from Bahrain, with 27% European haplotype, differs significantly from the samples from Norway and Sweden, by including a larger proportion of birds with Siberian haplotype (Table 2). There is a similar tendency for the sample from Italy, which also includes a larger proportion of Siberian haplotype than the Swedish sample (54% European haplotype, Table 2).

Dunlin from 17 localities were sampled in autumn (July–October), eight localities in spring (March–May) and four during winter (November–February, Table 1). No significant differences in haplotype frequencies between seasons could be detected in any country sampled during more than one season (Germany, Italy, Portugal, Spain, Sweden and Ukraine, χ^2, n.s. for all tests), however, in Germany and Sweden a tendency was found (χ^2, $P < 0.1$).

Table 3 Estimated average breeding longitudes of migrating and wintering dunlin and approximate distances to these areas

Sampling site	*N*	European haplotype (%)	Longitude (°E)	Distance (km)
Bahrain	26	27	112	6500
Germany	28	71	70	3600
Italy	217	54	87	5000
Kazakhstan	8	50	90	2500
Morocco*	26	100	—	3500
Norway	33	76	65	2400
Poland	20	55	86	3800
Portugal*	60	100	—	2800
Russia	16	37	102	1000
Spain	19	68	73	5300
Sweden	246	69	72	3200
Tunisia	20	35	104	7000
Ukraine	33	42	97	4400
Total	752			

*Portugal and Morocco have only European haplotype. This is typical of all studied populations of the subspecies *Calidris alpina schinzii*. They breed on Iceland, in Great Britain and around the Baltic, approximately between longitudes 24°W and 30°E. Distances for Portugal and Morocco refer to Icelandic breeding grounds.

Breeding origin and migration distances of migrating and wintering dunlin

By comparing the haplotype composition of migrating and wintering dunlin with haplotypes of breeding dunlin, it is possible to obtain an estimate of where the birds come from (Table 3). For samples including both European and Siberian haplotypes, the average breeding longitude is estimated

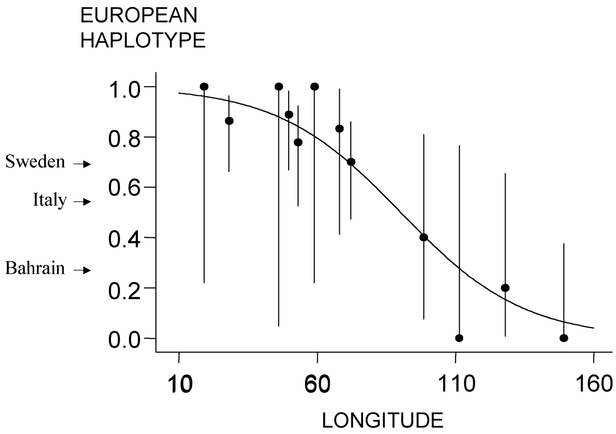

Fig. 3 Frequency of European haplotype in breeding dunlin as a function of breeding longitude. Vertical lines show the 95% confidence interval for the haplotype frequencies at each site. The sigmoid curve follows the logistic equation $y = 1 - 1/(1 + 58e^{-0.045x})$. The frequency of the European haplotype is weighted by the number of sampled birds at each longitude. Arrows on the left indicate the proportions of European haplotype in samples of migrating and wintering dunlin from Sweden, Italy and Bahrain, respectively.

using the relationship between the proportion of European haplotype and the breeding longitude shown in Fig. 3. It should be pointed out that this method provides only a broad estimate of the approximate *average* breeding longitude. It is not possible to tell whether a group of migrating or wintering dunlin all come from a rather restricted breeding area with the observed haplotype composition, or if the sample is a mixture of birds from a wide breeding area which together give the same total haplotype proportions.

At the breeding grounds, *C. a. schinzii* have only the European haplotype, with the possible exception of the south Norwegian population (Kålås & Byrkjedal 1981; Wenink *et al.* 1996), whereas all *C. a. alpina* populations seem to include both the European and Siberian haplotypes (Fig. 1). Thus, the analyses of the haplotype composition of 60 Portuguese and 26 Moroccan dunlin, showing 100% European haplotype, indicate that the majority of these dunlin belong to the subspecies *C. a. schinzii.* They breed mainly in Iceland, but are also found in smaller numbers in the British Isles and around the Baltic Sea (Cramp & Simmons 1983). The sample may also include dunlin of the subspecies *C. a. arctica* from Greenland, which also seem to have only the European haplotype (Wenink *et al.* 1996), and thus can not be distinguished from *C. a. schinzii* using this method. It may also include a smaller fraction of *C. a. alpina*, significantly less than 25% (χ^2, $P < 0.05$). However, as no birds with the Siberian haplotype were found, the proportion of *C. a. alpina* is likely to be low. In contrast to the Portuguese sample, the Spanish sample contains a relatively large proportion of birds with the Siberian haplotype which indicates that it includes mainly *C. a. alpina.*

The dunlin which migrate to north-west Europe originate mainly from breeding areas in the western Palaearctic. The regression of haplotype frequencies gives an estimated average breeding longitude of 71° E for the north European samples, which is on the Yamal peninsula in northern Russia.

Like those in north-west Europe, the Italian dunlin also originate mainly from breeding areas in north-west Russia. However, the haplotype frequencies indicate that, on average, their breeding areas tend to be further east, thus including more birds from central Siberian populations. The Italian sample has an estimated average breeding origin of 87° E, which is on the western part of the Taimyr peninsula. The estimated average breeding longitude for Bahrain is 112° E, which indicates that these birds come mainly from breeding areas on Taimyr and east thereof.

The distance from the sampling locality to the estimated average breeding areas is approximately 2800 km from Portugal, 3500 km for Morocco and 4000 km for north-west Europe. Further east the dunlin travel longer distances: 5000 km to Italy, 7000 km to Tunisia and 6500 km to Bahrain (Table 3).

Discussion

The mtDNA haplotypes of migrating and wintering dunlin can now be used, not only to identify to which phylogenetic group the birds belong, but also to estimate their breeding origin. The major DNA lineages have been identified by DNA sequencing, and now simple digestion of the PCR-product by specific restriction enzymes is enough to identify these phylogenetic groups. The risk of assigning an individual to an incorrect lineage due to a mutation in a restriction site has been shown to be extremely low (Wennerberg *et al.* 1999). The results are highly reliable and restriction analysis is faster and less expensive than sequencing, which makes the method convenient, especially for screening large sample sizes.

The distribution of haplotypes in the breeding populations of dunlin is based on data from 22 breeding sites in the Palaearctic (Fig. 1). The overlap of haplotypes between populations is extensive, but the haplotypes of dunlin are still clearly structured and the haplotype frequencies show a consistent geographical pattern that may serve as a basis for estimating the breeding origin of migrating and wintering dunlin in the Palaearctic.

The mtDNA results show that dunlin breeding in the western and central Palaearctic are found in at least four different geographical regions during the winter. These areas may often contain mixes of dunlin from more than one breeding population, and individuals from the same breeding area may be found in different winter regions. However, the winter areas show different haplotype composition, revealing differences in breeding origin. Morphological data and ringing recoveries, as well as information about, for example, the timing of migration and moult support the groups detailed below.

The Atlantic coast of south-west Europe and north-west Africa

This group was sampled on migration in Portugal and Morocco, where all birds had the European haplotype. This indicates that they originate mainly from Iceland, but dunlin from other breeding areas may also be involved in smaller numbers. Ringing recoveries, as well as morphological data, also show that Portugal and Morocco are situated on the main migration route of Icelandic dunlin (Pienkowski & Dick 1975; Hardy & Minton 1980; Sandberg & Gudmundsson 1996). There are a few recoveries of Baltic *Calidris alpina schinzii* (which also have 100% European haplotype) in west Africa, but they are recovered mainly on wintering areas further north (e.g. in France, Jönsson 1986). A low proportion of *C. a. alpina* is also likely involved, as indicated by, for example, ringing recoveries from Russia and the Baltic (Greenwood 1984; Pettersson *et al*. 1986).

In winter, about 45 000 dunlin stay in Portugal (Costa & Rufino 1997; Snow & Perrins 1998). However, most birds continue to migrate to wintering areas on the west coast of north Africa, where the estimated number of dunlin ranges between 220 000 and 675 000 birds according to Pienkowski & Dick (1975), and the estimate of 900 000 dunlin at Banc d'Arguin (Zwarts *et al.* 1997) increases the numbers further.

North-west Europe

Dunlin caught on migration in north-west Europe had around 70% European haplotype, which corresponds to an estimated average breeding longitude of 71° E. This area is located on the Yamal peninsula in northern Russia. The dunlin wintering in north-west Europe surely include birds from Yamal, as well as a mix of dunlin from populations both west and east of this longitude. Ringing recoveries extend from northern Scandinavia in the west to Taimyr peninsula in the east (Greenwood 1984; Liljefors *et al*. 1985; Gromadska 1989).

Most of the dunlin probably stay in this area during winter. Recoveries of birds caught on migration in Sweden, Norway and Germany are most often found in Britain during winter (Hardy & Minton 1980; Roos 1984; Pettersson *et al*. 1986; Rösner 1997), where more than 500 000 dunlin spend the winter (Tucker & Heath 1994; Pollitt *et al.* 2000). Many recoveries also originate from wintering grounds in France and the Wadden Sea, which also host large numbers of dunlin during winter (France, 240 000; the Netherlands, 181 000; Germany, 85 000; Snow & Perrins 1998), and some recoveries link the north-west European migrants to wintering areas in southern Europe and north Africa.

The haplotype composition of the Polish birds indicates a more eastern breeding origin on average than for the other north European samples. The large proportion of birds that have initiated their primary moult before arriving in Poland, as well as their 'adult buff' coverts, also indicates that central Siberian populations may be included, in contrast to British wintering dunlin, where these phenomena are uncommon (Gromadska 1989). Ringing recoveries of Polish dunlin show links with, for example, Britain and the Netherlands, as well as with more easterly wintering areas in the Mediterranean and the Black Sea (Gromadska 1989; Serra *et al*. 1998).

The Mediterranean region

Italian dunlin have about equal proportions of European and Siberian haplotypes. This haplotype composition tends to differ from the composition in northern Europe (Sweden). However, many of the Italian ringing recoveries come from this region (mainly Poland and Sweden), linking the Baltic and Mediterranean (Gromadska 1989). The occurrence of recoveries from the east (Ukraine, Czech Republic, Slovenia and Hungary) has suggested that there is also an influx of dunlin from central Siberia (Serra *et al*. 1998). This is now supported by mtDNA data. About 15 000 dunlin winter in Italy (Snow & Perrins 1998), but many of the Italian migrants probably head for wintering grounds in Tunisia, where several ringing recoveries have been found (Serra *et al*. 1998).

Middle East

This group was sampled during migration in Bahrain (and possibly also in Kazakhstan), having mainly the Siberian haplotype, but still including a significant proportion of European haplotype and no birds of Beringian (or Alaskan) haplotype. The sample from Bahrain has an estimated average breeding origin of 112° E. This haplotype composition suggests that they come mainly from breeding areas on the Taimyr peninsula in central Siberia, or east of there, but not as far east as the breeding areas of *C. a. sakhalina*. A similar haplotype composition was found for eight birds from the same area analysed by Wenink & Baker (1996). Large numbers of dunlin stay in the Persian Gulf for the winter, whereas others continue even further south, to wintering areas in for example the Red Sea (Cramp & Simmons 1983).

Beringian and Alaskan haplotypes were not found at all among the migrating and wintering birds. This shows clearly that the dunlin populations breeding in east Siberia do not migrate to these areas. Single European migrants are sometimes attributed to the east Siberian subspecies *C. a. sakhalina* (e.g. Cramp & Simmons 1983), but the results show that the mitochondrial gene flow from east Siberian populations to the populations wintering in the western Palaearctic is probably negligible. mtDNA is inherited maternally, but nucleic DNA can be expected to show similar results, as male dunlin are at least as site-faithful as females (Soikkeli 1970; Thorup 1999). Dunlin with the Beringian haplotype breed only in eastern Siberia (Fig. 1, Wenink *et al*. 1996). They have not been found on migration anywhere in the western Palaearctic, but have been sampled on migration in Hong Kong, as well as on the west coast of North America (Wenink & Baker 1996). Birds of Alaskan haplotype, which breed mainly in Alaska, and have also been found on Wrangel Island in eastern Siberia, are also recorded on migration on both sides of the Pacific Ocean (Wenink & Baker 1996; Wenink *et al*. 1996; Wennerberg *et al*. 1999).

Different populations of dunlin may visit the same site at different times of the year, as, for example, in Britain where *C. a. schinzii* occur in large numbers of migration, but *C. a. alpina* is by far the most common subspecies in winter (Hardy & Minton 1980). Therefore, time of year was taken into account in the discussion of the migration patterns, even though no significant differences in haplotype frequencies between seasons were detected in this study. Particularly, if sample sizes are low and frequency differences small, such variation is difficult to detect. Tiedemann (1999) argued that the German migrants included more birds of the subspecies *C. a. schinzii* in autumn than in spring based on mtDNA sequence variation, but the difference between the seasons was not tested statistically. The German samples analysed here do not confirm this suggestion. However, the number of samples from Germany is low in both studies and no clear conclusion of the relative occurrence of the two subspecies can therefore be drawn.

The migration system of dunlin in the western Palaearctic, as reflected by mtDNA analysis

The results of mtDNA analyses show that the migration system of the dunlin in the Palaearctic can, in general, be considered to be a parallel migration system (Salomonsen 1955). Populations from the westerly part of the breeding range winter further to the west, and those breeding successively further to the east also winter further east. The shift in haplotype frequencies on the breeding grounds, from 100% European haplotype in western Europe to including more and more Siberian haplotype in central Siberia, is reflected by a similar shift at the wintering grounds from west (Morocco) to east (Bahrain). Parallel migration is also found in the Nearctic dunlin. The subspecies *C. a. hudsonia*, breeding in central Canada, winters on the east coast of North America and in the Mexican Gulf, whereas the more western *C. a. pacifica*, breeding in western Alaska winters mainly on the west coast of North America (Warnock & Gill 1996).

The different types of migration systems have been characterized by Salomonsen (1955). Parallel migration systems are common among birds. Other examples are the rufous bush robin *Cercotrichas galactotes* and the lesser black-backed gull *Larus fuscus* (Salomonsen 1955).

Even if a parallel migration system seems to be the general pattern in the dunlin, the species also show aspects of chain migration (Nilsson 1858; Lundberg & Alerstam 1986) as well as leap-frog migration (Palmén 1874) in parts of the species' range. These systems may evolve in different situations, depending on, for example, habitat suitability, dominance and cost of migration (Lundberg & Alerstam 1986; Holmgren & Lundberg 1993).

On the west coasts of Europe and Africa, the two subspecies *C. a. schinzii* and *C. a. alpina* seem to show a system of chain migration. Populations of *C. a. alpina*, which breed furthest to the north on the Scandinavian and Russian tundra, often winter furthest north (mainly in north-west Europe), whereas *C. a. schinzii* populations, breeding more southerly, in Iceland, Britain and the Baltic region, mainly winter further south (in west Africa and south-west Europe).

According to Salomonsen (1955), there are two types of leap-frog migration systems: the 'longitudinal type', in which the populations migrate along approximately the same longitudes, and the 'parallel type', where northern breeding populations winter further south, but do not migrate past the more southern breeding areas. Dunlin populations in Russia and central Siberia may be considered to show the second type of leap-frog system. The populations in western Russia winter mainly in north-west Europe, whereas those breeding further north in central Siberia tend to migrate to more southerly wintering areas (e.g. Tunisia, Bahrain).

The distances, along loxodromes (Gudmundsson & Alerstam 1998), between the breeding and wintering areas of dunlin in the western and central Palaearctic increase eastwards in this area. The shortest migration distances (2500–3000 km) are those of *C. a. alpina* from northern Scandinavia and north-western Russia that winter in north-west Europe, as well as for Baltic *C. a. schinzii* wintering on the European west coast. However, the birds caught on migration in north-west Europe are estimated to have an *average* breeding origin of 71° E, which corresponds to a migration distance of approximately 4000 km. Dunlin from Iceland, wintering in north-west Africa, migrate distances of 3500–5000 km. Dunlin wintering further east have even longer distances to travel, e.g. about 5000 km for the Italian sample, 7000 km to Tunisia and 6500 km to the stopover site in Bahrain.

In most cases, these calculated migration distances are probably somewhat shorter than the actual distance travelled by the birds. In some cases, the birds may follow leading lines (e.g. rivers or coast lines) resulting in a longer migration distance (Palmén 1874; Bingman *et al*. 1982; Åkesson 1993; Gudmundsson 1994). Birds may also head for good feeding or moulting sites on the way, as fat stores from the breeding areas are generally too small to take them all the way to the wintering grounds (Lindström 1998; Å. Lindström, M. Klaassen, T. Piersma, N. Holmgren & L. Wennerberg, unpublished manuscript). The parallel migration system often gives the dunlin the shortest distances to cover on migration between breeding and wintering areas, resulting in both time and energy minimization. Variation in habitat quality, especially at the wintering ground, and the advantages of early arrival at the breeding areas may also be important factors in the evolution of longitudinal segregation (Lundberg & Alerstam 1986). However, the migration system may, at least to some extent, also have an important historic component, and migration routes today may resemble those that existed during for example the latest ice age, when the breeding areas were altered, as the climate influenced the range of suitable breeding habitat. For example, the east–west separation between European and Siberian haplotypes may result from times when these groups were found in geographically separated regions, using different migration routes.

Conclusions

Distinct population markers may be difficult to find for dunlin and most other wader species with more or less continuous breeding habitat, as gene flow between populations reduces the genetic differentiation among them. Instead, differences in allele frequencies between populations can be used to identify the origin of birds on migration (as in this study). The method may be improved further by including more genetic markers that differ in frequency between populations. Thereby, studies of other variable parts of the genome, for example intron sequences or microsatellites, which are highly variable, may give information about the origin of migrating waders on a finer geographical scale.

The results of this study show that genetic markers can be used as a valuable tool in studies of bird migration for identifying morphologically similar populations, and for recognizing them in different geographical areas during different parts of their annual migration cycle. Particularly in combination with other population parameters, such as morphological and behavioural variation and recapture data, this may give many new and interesting possibilities for studies of migration of all types of organisms, making it possible to study selection pressures and adaptations to the different environments throughout the migration cycles.

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