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Reconstructing palaeoflyways of the late Pleistocene and early Holocene Red Knot *Calidris canutus*

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Bird migration systems must have changed dramatically during the glacial–interglacial cycles of the Pleistocene and as novel habitats became available since the last glacial maximum. This study combines molecular dating of population divergence times with a review of polar-centred palaeovegetation and intertidal habitats world-wide to present a hypothesis for the evolution of Red Knot *Calidris canutus* flyways. Divergence dates from coalescent analysis of mitochondrial control region sequences indicate that *C. c. canutus* diverged from the most recent common ancestor (MRCA) of Red Knots about 20 000 (95% CI 60 000–4000) years ago. About 12 000 (95% CI 45 000–3500) years ago this MRCA diverged into two lineages, now represented by the North American breeding *C. c. roselaari*, *C. c. rufa* and *C. c. islandica* and the Siberian breeding *C. c. piersmai* and *C. c. rogersi*, respectively. Divergence times of these two Siberian breeding subspecies are about 6500 (95% CI 25 000–1000) years ago, and populations of the North American breeding subspecies are estimated to have diverged within about the last 1000 years. These divergence times suggest that all ancestral populations of knots emerged within the last glacial period of the Pleistocene via an eastward expansion into North America. This scenario implies that, contrary to contemporary opinions, *C. c. islandica* was not recently derived from *C. c. canutus* despite the fact that they are morphologically similar and that their contemporary migration routes overlap in the Wadden Sea. Instead, *C. c. islandica* is most closely related to the other North American breeding subspecies *C. c. roselaari* and *C. c. rufa*. Thus, *C. c. islandica* only recently pioneered its current migration route to Europe, following the amelioration of winter conditions in the Wadden Sea and the formation of staging habitat in Iceland. This implies that, in Red Knots at least, the Greenland/Iceland migratory route was established very recently from breeding grounds in the Americas to wintering grounds in Europe and not *vice versa* as previously believed.

Key words: Red Knot, *Calidris canutus*, generation time, evolution, migratory routes

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INTRODUCTION

During the glacial-interglacial cycling of the Pleistocene and as current habitats became available since the last glacial maximum, bird migration systems have changed dramatically (Alerstam 1990). Early paleogeographic studies on bird migration were interested in the effect of ice-free refugia on the present day distribution and morphological patterns of bird populations (Larson 1957, Ploeger 1968). However, the Quaternary ice ages have also left a genetic legacy (Hewitt 2000), and with the advent of molecular technology, to date population divergences, other studies have emphasized the importance of Pleistocene glaciations on population structure and hypothesized ways in which current population distributions might have arisen in shorebirds (Piersma 1994, Wenink *et al.* 1996, Kraaijeveld & Nieboer 2000) and passerines (Klicka & Zink 1997, Johnson & Cicero 2004, Zink *et al.* 2004, Lovette 2005). However, there have been no attempts to incorporate both molecular dating and an in-depth review of palaeovegetation and coastal geomorphology to examine the recent evolution of bird flyways in detail.

To examine the evolution of a flyway system for a particular species several criteria must be met. First, detailed knowledge of the specific habitat requirements of the species is needed. Second, these habitats should be identifiable in the fossil/ geomorphic record to allow a reconstruction of past habitat distribution. Finally, data should exist over a sufficient scale to allow sensible reconstruction with reference to the global movements of the species (in the case of long distance migrants).

Red Knots *Calidris canutus* fulfil many of the requirements for an examination of flyway evolution, at least for the late Pleistocene and Holocene periods. Knots are divided into six subspecies on the basis of plumage colour, body size and migratory route: *C. c. canutus,* C. c. *piersmai,* C. c. *rogersi,* C. c. *roselaari,* C. c. *rufa* and C. c. *islandica* (Tomkovich 1992, 2001). Three of these subspecies are also genetically distinct on the basis of mitochondrial DNA (C. c. *canutus,* C. c. *piersmai,* C. c.

rogersi) and a fourth lineage is comprised of C. c. *roselaari,* C. c. *rufa* and C. c. *islandica* (Buehler & Baker 2005). Overall, knots have very low genetic diversity both in the mitochondrial (control region) and nuclear (microsatellites and amplified fragment length polymorphisms AFLPs) genomes and although broad scale sequencing of nuclear genes may uncover structure among C. c. *roselaari*, C. c. *rufa* and C. c. *islandica* in the future, such structure has so far remained elusive (Buehler 2003).

Knots are selective feeders that use a specialized sensory mechanism to locate hard-shelled prey buried in soft sediments (Piersma *et al.* 1998). Thus in the non-breeding season, they are confined to intertidal coastal habitats with extensive stable soft sedimentary habitats (Piersma 1994, Piersma *et al.* 2005). In the breeding season they are cold tundra specialists and are thus confined to the High Arctic (Piersma & Davidson 1992). Both intertidal sediments and High-arctic tundra habitats are identifiable in the geological record and have been studied in many parts of the world. In the case of High-arctic tundra habitat, some studies have even generated comprehensive global summaries. Furthermore, arguments from first principals about sea level changes may allow us to generalize over gaps in the intertidal record.

Hypotheses regarding the timing of the population bottleneck in Red Knots have been proposed (Piersma 1994, Kraaijeveld & Nieboer 2000). Piersma (1994) posited that the bottleneck occurred during the Holocene deglaciation when boreal forest reached up to the ice sheets and intertidal areas may have been flooded by rising sea levels, caused by the melting of the ice sheets, at a rate too fast for the formation of stable intertidal sediments, which sustain the bivalve populations necessary for knots during the non breeding season. Kraaijeveld and Nieboer (2000), however, argued that breeding habitat for Red Knots was restricted during the climatic optimum in the early Holocene (7000 to 8000 years ago) much more so than during deglaciation, and thus the climatic optimum rather than deglaciation coincided with the population bottleneck in knots (Kraaijeveld & Nieboer 2000). To test these hypotheses this study inte-

grates molecular estimates of population divergence time and a review of palaeovegetation for both cold tundra and intertidal areas, for the late Pleistocene and Holocene periods.

Habitat requirements for Red Knots

Red Knots are one of the most polar of all High-arctic breeders. They reproduce only on the northern most fringes of land, often within sight of the Arctic Ocean. In this habitat vegetation cover is sparse, usually between 4 and 25% (Olson *et al.*, 1983), and conditions are harsh even in the summer (Piersma *et al.* 2003). On the tundra they eat mostly spiders and other arthropods which they obtain by surface pecking (Tulp *et al.* 1998). Outside the breeding season knots occur only on coastal sites with large expanses of intertidal substrate, and all current wintering habitats for knots are confined to marine intertidal areas with stable sediments and hard-shelled prey. Estuaries such as the Wadden Sea and the Wash in northwest Europe, and Bahia Lomas in Chile all contain extensive mudflats and an abundance of hard-shelled prey including *Macoma balthica, Mytilus edulis* and *Hydrobia ulvae* (van Gils *et al.* 2005). Red Knots are extremely specialized feeders that use a unique bill-tip organ to locate hard objects in soft sediments (Piersma *et al.* 1998). Hard-shelled bivalves buried in the soft yet stable mud of intertidal flats are the ideal prey for knots and are ingested whole and crushed in a muscular gizzard (Piersma *et al.* 1999, Battley & Piersma 2005). This specialized method of feeding, along with possible peculiarities in their immune system (Piersma 1997, 2003), may restrict Red Knots to very specific non-breeding habitats.

An exception to the habitat and diet restrictions described above occurs during northward migration at a stopover site in Delaware Bay, USA. There, Red Knots do not need to use their specialized feeding technique to locate buried bivalve prey because the beaches are covered with a nutritious and easy to find food source. In Delaware Bay *C. c. rufa* and *C. c roselaari* subspecies feed on Horseshoe crab *Limulus polyphemus* eggs. There are four extant species of horseshoe crab, of which

Tachlypeus tridentatus, Tachlypeus gigas and *Carcinoscorpius rotundicauda* are all found in the coastal waters of southeast Asia including the Philippines and Indonesian (35°N to 5°S), while *Limulus polyphemus* inhabits the waters of the western Atlantic Ocean from Maine to the Yucatan (44.5°N to 21°N). Little is known about shorebird and horseshoe crab interactions in the Indo-Pacific. However, the dependence of shorebirds on horseshoe crab eggs during spring migration in Delaware Bay has been well established (Tsipoura & Burger 1999, Schuster *et al.* 2003, Baker *et al.* 2004).

The temporal framework

Population structure and genetic diversity in Red Knots has been well studied using molecular techniques. Mitochondrial DNA, microsatellites and AFLP data have shown that knots have very little genetic diversity, and because this lack of diversity occurs across the genome, these data support the proposal that knots have undergone recent and severe population bottlenecks (Buehler 2003). The current hypothesis is that population bottlenecks occurred during the last glacial of the Pleistocene 115 000 to 10 000 years ago, as well as during the Holocene warming. Buehler & Baker (2005) proposed that *C. c. canutus* diverged from the most recent common ancestor (MRCA) of Red Knots about 20 000 (95% CI: 60 000–4 000) years ago. The MRCA of the lineage leading to *C. c. roselaari*, *C. c. rufa* and *C. c. islandica* subspecies was dated at about 12 000 (95% CI: 45 000–3500) years ago and the split between *C. c. piersmai*, and *C. c. rogersi* at about 6500 (95% CI: 25 000–1000) years ago. These population divergences were estimated using a computer program MDIV based on a coalescent model that jointly estimates the divergence time and migration rates among pairs of populations using DNA sequence data (Nielsen & Wakeley 2001). The method estimates several parameters using Markov Chain Monte Carlo simulations to generate posterior probability distributions. The 95% credibility intervals associated with the dates represent the interval that contains 95% of the posterior probability distribution.

Buehler & Baker (2005) used age of first reproduction (2 years) as an estimate for generation time in Red Knots. However, an improved calculation for generation time has recently become available. This new calculation takes into account the effect of adult survival on generation time and is calculated as follows: $g = \alpha + [\ s\ /\ (1–s)\ ]$ where α is age of first reproduction and s is expected adult survival rate (Lande *et al.* 2003, Sæther *et al.* 2005). In a long-lived shorebird species such as the Red Knot an individual's contribution to the gene pool may be affected by its survival, thus we recalculated generation time for Red Knots as $g = 2 + [0.8\ /\ (1 – 0.8)] = 6$ years, using an estimate of 0.8 for adult survival (Brochard *et al.* 2002). Because mutation rate per year is unaffected by generation time this new calculation does not change the population divergence time estimates given by Buehler & Baker (2005). However, a generation time of six rather than two years reduces the estimates of effective population size threefold, strengthening the argument for a recent and severe population bottleneck in Red Knots.

In this paper we extend the hypothesis of Buehler & Baker (2005) by including a more detailed examination of Red Knot habitats during emergence of present day flyways and by considering how these habitats may have impacted seasonal migrations and long term range expansions. Finally, we reflect on whether Red Knot flyways might be representative of other High-arctic waders and how this relatively rapid evolution of flyways argues against strict genetic programming of migration in waders.

A SCENARIO FOR THE EVOLUTION OF RED KNOT FLYWAYS

Eastward expansion from Eurasia into North America

Figure 1 summarizes the current distribution of Red Knots and overlays on it a phenogram that summaries Red Knot population structure and the broad temporal framework presented above. Using these time periods as a guideline, we now present a scenario for the evolution of Red Knot flyways.

The Wisconsinan/Weichselian glacial from 115 000 to 10 000 years ago was characterized by many stadial and interstadial intervals of varying intensity and duration. Two cold and dry maxima occurred, one about 70 000 years ago and another 22 000 to 18 000 years ago. Between these two maxima conditions were more moderate and tundra habitat suitable for Red Knots was characteristic of northwest and central Europe (Bohncke 1993, Walker *et al.* 1993), northern Siberia (Serebryanny & Malyasova 1998, Hahne & Melles 1999), and small areas in northern Canada and Greenland (Möller *et al.* 1994, Lundqvist & Saarnisto 1995, Funder *et al.* 1998). Evidence also suggests that tundra existed south of the North American ice sheets, but this tundra may have been boreal in character and thus unsuitable for High-arctic waders such as Red Knots (Morgan *et al.* 1983). The area of arctic tundra during this time was greater than it is today and Red Knots were probably expanding their ranges (Kraaijeveld & Nieboer 2000). The last glacial maximum 22 000 to 18 000 years ago, however, was characterized by severe cold and aridity. During this period polar desert, characterized by <4% vegetation cover and thus a harsher habitat than used by Red Knots today, separated the East Asian tundra along the Kolymskij Mountains (Adams & Faure 1997). This polar desert may have split a single ancestral population of knots into two groups, one breeding west of the polar desert somewhere in central Siberia and one breeding in Beringia (Fig. 2A).

Current continental shelf depths, estimates of sea level change, and the location of large rivers that deposit large quantities of sediment in to the intertidal zone, can be used to make hypotheses about where suitable intertidal areas may have occurred during the last glacial maximum. Sea levels during the last glacial maximum have been estimated as 130 m lower than current sea levels (Pirazzoli 1996). As continental shelves range from 0 to 200 m below current sea level, intertidal areas may have existed at the edge of broad shelves. Furthermore, large rivers may have provided a constant source of intertidal sediment. We hypothesize that the Red Knot ancestral popula-
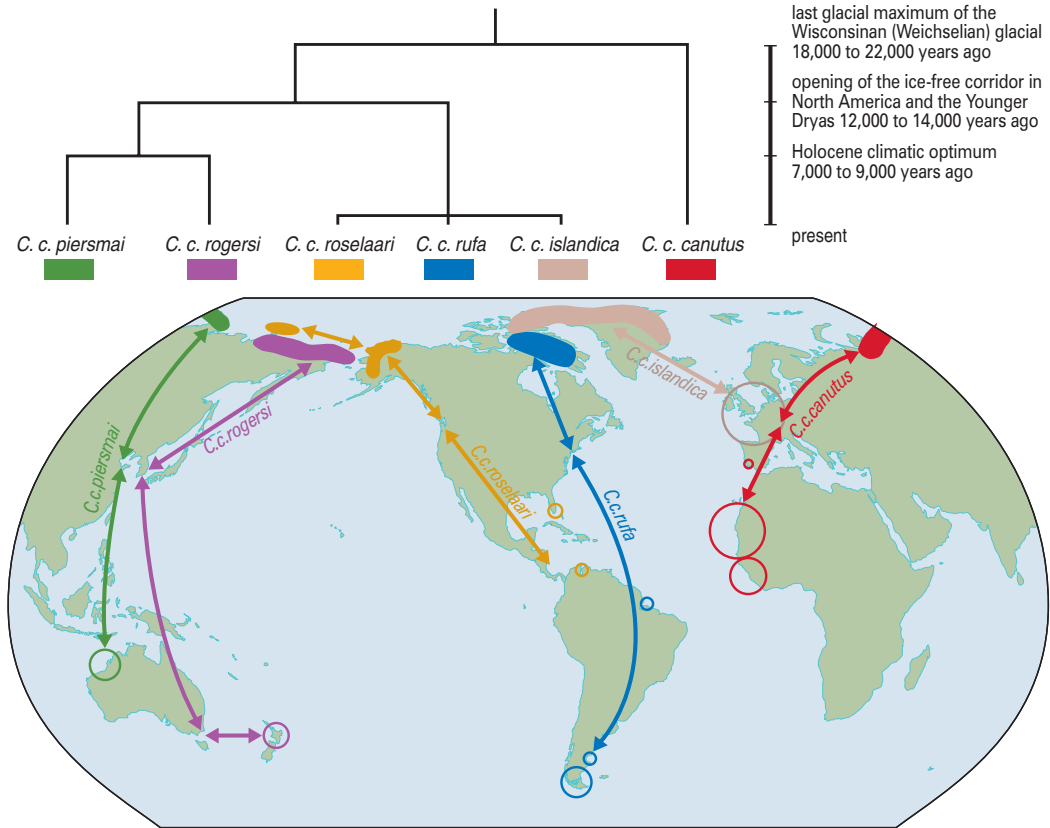
Figure 1. The global distribution of Red Knots (Piersma and Davidson 1992, T. Piersma pers. obs.). Shaded areas in the Arctic indicate breeding areas and circles indicate wintering areas. The size of the circle indicates the relative number of birds using the area. Migratory routes are colour coded to subspecies. Projected above the contemporary distribution of knots is a phenogram summarizing knot population structure and indicating major climatic events (with their approximate dates) that correspond roughly to divergence dates based on coalescent analysis of mitochondrial DNA. For population divergence date estimates and confidence intervals see text.

tion breeding to the west of the polar desert may have staged in the Mediterranean Sea either at the inflow area from the Black Sea or at the mouth of the Nile River. Although the Mediterranean does not have large tidal ranges, contemporary knots are known to stage in areas with small tidal ranges provided there is sufficient food available, the Kneiss area in Tunisia being a good example (van der Have *et al.* 1997). This ancestral population probably wintered in areas along the west coast of Africa as *C. c. canutus* does today, but the exact location of these wintering grounds during the last

glacial maximum is not known. Knots breeding in Beringia may have used the mouth of the Yangtze River and the Yellow Sea shores in China and the Koreas as a staging area and then continued south to wintering areas in Australia when the Yellow Sea shoreline systems froze over in winter.

Initial warming into our present interglacial began shortly after the last glacial maximum and gradually melted the ice in North America, thereby establishing an ice free corridor between the Laurentide and the Cordilleran ice sheets approximately 12 000 to 14 000 years ago (Pielou 1991,

Adams & Faure 1997). During this initial warming period we hypothesize that the Beringian population of Red Knots expanded its range east into North America (Fig. 2B). Beringia has been used as a route into North America by a great number of species, including Humans *Homo sapiens* (Shutler 1983). Once in North America, these birds may have used the ice-free corridor between the Laurentide and Cordilleran ice sheets to pioneer a new and shorter migratory route to wintering grounds at the mouth of the Mississippi River on the Gulf of Mexico, and may also have migrated to the west of the Cordilleran ice sheet (Fig. 2B). Today a major North American flyway runs nearly parallel to the former ice free corridor following the remnants of giant melt water lakes formed as the glaciers retreated. This hypothesis may explain why birds using this flyway do not follow a more straightforward migration route to breeding grounds in the central Canadian Arctic from the Gulf shores. Similar indirect migration routes have been attributed to Late Pleistocene range expansion, for example, in Swainson's Thrush *Catharus ustulatus* (Ruegg & Smith 2002). Finally, rapid cooling during the Younger Dryas (also about 12 000 years before present) may have played a role in isolating the Beringian and North American ancestral populations. During the Younger Dryas, polar desert crept south and may have geographically split the breeding populations (Fig. 2B).

Eurasia reached the climatic optimum of the current Holocene interglacial about 7000–9000 years ago (Adams & Faure 1997), during which woodland crept northward, reaching 300 to 400 km further north than its current boundary. Larches *Larix* spp. and birch *Betula* spp. trees were found in tundra in much of northern Siberia (Kremenetski *et al.* 1998) and shrub tundra covered northern Taimyr (Hahne & Melles 1999). In North America boreal forest reached right up to the remaining Laurentide ice sheet (Roberts 1989). Thus the Holocene optimum caused a large decrease in tundra breeding habitat and probably initiated local population bottlenecks in Red Knots. However, unlike Kraaijeveld and Nieboer

(2000), we think it is unlikely that Red Knots were bottlenecked to a single refugial population in northern Canada and Greenland during the optimum. The subspecies currently inhabiting the High Canadian Arctic and Greenland has extremely low levels of nucleotide diversity making this area unlikely as the refuge for the ancestral population of Red Knots (Buehler & Baker 2005). Rather we propose that before the climatic optimum the Beringian breeding MRCA of *C. c. piersmai* and *C. c. rogersi* expanded its range into the Chukotka Peninsula. Later, during the climatic optimum, suitable tundra areas were isolated from one another by forest possibly causing the split between the *C. c. piersmai* and *C. c. rogersi* (Fig. 2C). We hypothesize that both populations probably followed migratory routes similar to what they do today using intertidal areas in the Yellow Sea and tropical Australia, (Battley *et al.* 2005) although the exact details of these routes will probably remain unknown.

A new migratory route into Europe
The most intriguing finding in this study is the possible establishment of the Greenland/Iceland migratory route from breeding grounds in North America to wintering areas in Western Europe. This idea contradicts conventional wisdom, which argues that this route most likely evolved via a northward range expansion into Iceland and then Greenland from staging and wintering areas in Western Europe. In this scenario, *C. c. canutus* and *C. c. islandica* are predicted to be sister subspecies (Piersma 1994). This idea has been supported in the past by morphological data which show *C. c. canutus* and *C. c. islandica* as similar in size and plumage colour (Tomkovich 1992), and very similar in their timing of annual cycle events including moult (Piersma & Davidson 1992). However, data presented in Buehler & Baker (2005) show *C. c. islandica* as genetically almost indistinguishable from *C. c. rufa* and *C. c. roselaari* birds. Furthermore, regardless of the exact dating, the North American breeders are deeply split from *C. c. canutus*. This distant relationship of *C. c. canutus* and *C. c. islandica* supports the hypothesis of an
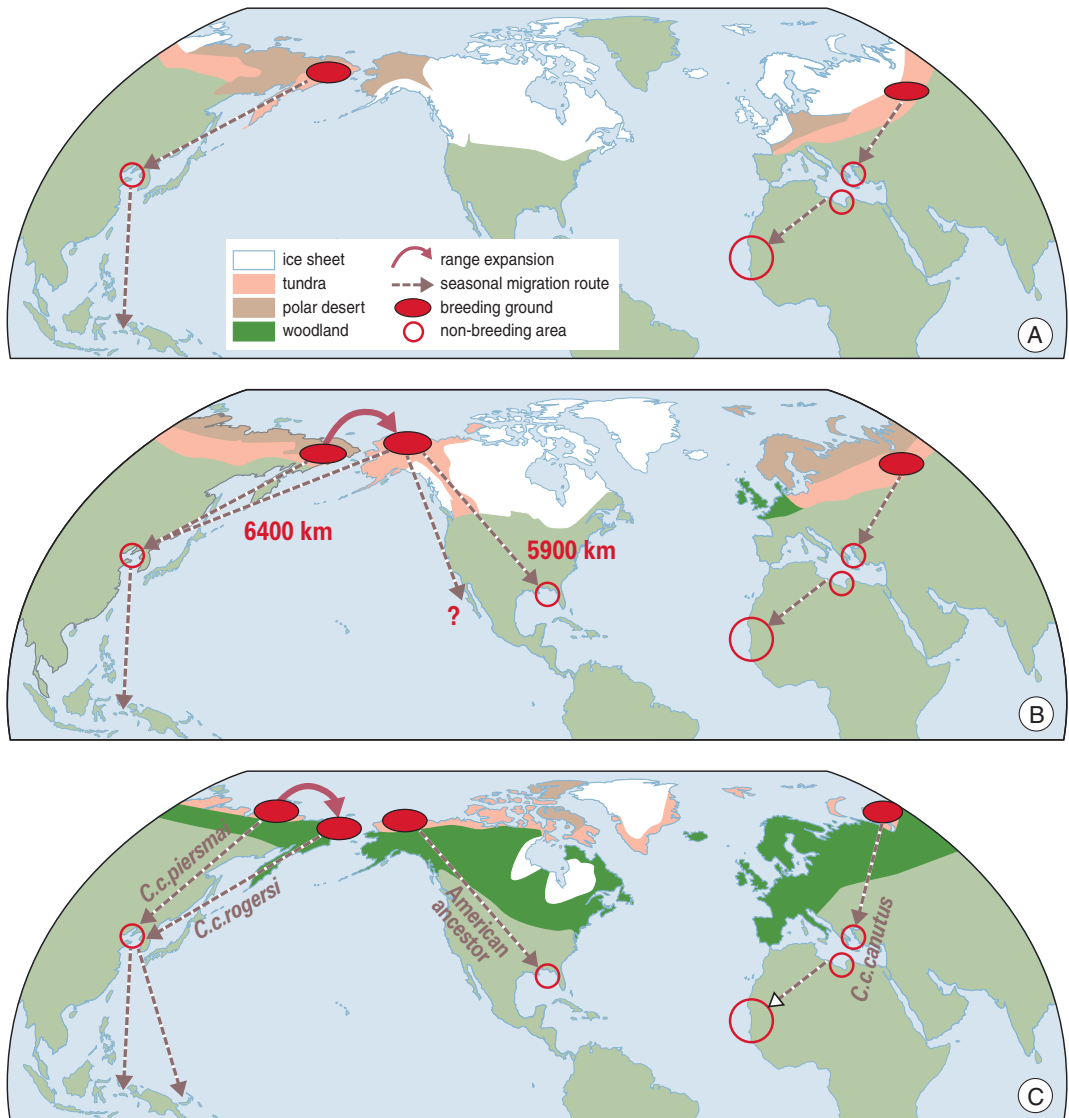
Figure 2. A graphical representation of our scenario for the evolution of Red Knot flyways. Palaeoreconstructions have been compiled from Adams and Faure (1997) and span from the last glacial maximum 18 000 to 20 000 years ago to present. In this figure polar desert refers to habitat characterized by <4% vegetation cover, thus harsher than the 'polar desert' used by Red Knots today in which vegetation cover is usually between 4 and 25%.

(A) Possible knot distribution during the last glacial maximum 18 000 to 20 000 years ago. Woodland is not considered at this time point. (B) Possible expansion of knots into the North American Arctic. The great circle distances for the competing migratory routes are indicated. (C) Possible expansion of knots into the Chukotka Penninsula just before the Holocene climatic optimum, and the survival of knots in four isolated refugia during the optimum when suitable breeding tundra was greatly reduced (7000 to 9000 years ago).

expansion of knots across the North American Arctic from Beringia and the establishment of a new migratory route from the breeding grounds into Europe.

External morphological and plumage colouration similarities between *C. c. canutus* and *C. c. islandica* could have been acquired convergently by local adaptation on the breeding grounds, as both subspecies experience similar breeding conditions in the High Arctic of Northern Canada, Greenland and the Taimyr Peninsula. Similarities in winter plumage and the timing of moult could also have been acquired convergently as these subspecies partially share the non-breeding grounds as well. The environmental conditions that these two subspecies experience differ from those experienced by *C. c. rufa* and *C. c. roselaari*, which occupy more dissimilar breeding ranges and wintering habitats. Indeed, this emphasizes that, when attempting to reconstruct population divergences, care should be taken to choose selectively neutral markers so that homologous and not convergent evolution can be examined. Genes under selection might be more likely to reveal genetic differences between the subspecies, but because of the possibility of convergence they would not represent historical population divergences. In this respect, we argue that the molecular markers used in Buehler & Baker (2005) may better represent the actual population structure of Red Knots than morphological characters.

Molecular markers give only an estimation of population divergence times, and for the mitochondrial DNA estimates presented in Buehler & Baker (2005) credibility intervals for the population divergence of *C. c. roselaari, C. c. rufa* and *C. c. islandica* include zero (95% CI: 0–16 500 years ago). Thus, we can only conclude that these populations diverged at some point between the last glacial maximum and the present. Contemporary Red Knots require cold tundra with sparse vegetation for breeding and extensive intertidal areas with soft sediments during the non-breeding season. Assuming that these current habitat requirements are representative of what was needed for survival in the past, we can now

ask where these habitats occurred during the last glacial maximum and the subsequent Holocene warming.

Tundra vegetation has only recently colonized land left barren by receding ice sheets in the Canadian Arctic, allowing Red Knots to expand their breeding range east from northern Alaska. We propose that this eastward expansion of breeding ranges and the emergence of present day intertidal areas facilitated the evolution of the current flyways of *C. c. rufa* and *C.c. islandica*. How did *C. c. rufa* pioneer a migration route into the southern most reaches of South America? These birds were probably expanding their range eastwards in the central Canadian Arctic as the ice sheets receded and tundra became available. As they migrated south they would have found suitable wintering habitats in the south eastern parts of North America already occupied by the earlier evolved C. c. roselaari population, possibly necessitating a flight across the Gulf of Mexico (Fig. 3A). In addition, the prevailing wind conditions may have played a role, with knots occasionally blown over the Atlantic Ocean during a season with particularly strong westerly winds. Once over the open water, individuals who had made it far enough south might have been aided by easterly equatorial trade winds facilitating migration to South America (Stoddard *et al.* 1983, Gauthreaux 1991, National Geographic Society 1992). In South America suitable staging areas may have existed at and around the mouth of the Amazon River, and by about 5000 years ago intertidal areas in Tierra del Fuego also became available for knots (Porter *et al.* 1984, Gordillo 1999).

The question then becomes how did the birds that wintered in South America return to their breeding grounds and where could they stage on spring migration? Birds of the *C. c. rufa* subspecies currently rely on spawning Horseshoe crabs in Delaware Bay to provide them with abundant food for their journey to the Arctic. The sandy beaches along the north eastern seaboard of North America became suitable for Horseshoe crab spawning very recently and it is possible that huge numbers of crabs were not found in the area until a few thou-

sand years ago (Schuster *et al.* 2003). This emergence of the North American seaboard as a staging area may have provided *C. c. rufa* pioneers with the stepping stone that they needed to reach breeding tundra in the Canadian Arctic (Fig. 3A).

As ice-free habitat emerged in the High-arctic islands of Canada and Northern Greenland, Red Knots currently considered *C. c. islandica* probably expanded their breeding range to the north and east (Fig. 3C). Genetic analysis of Greenland Eskimos also indicates relatively recent expansions into these areas (Saillard *et al.* 2000). But how might individuals of the *C. c. islandica* subspecies have pioneered a migratory route to wintering areas in Europe? The proximity of High-arctic breeding areas to the North Pole means that only a small eastwards shift in orientation (50°) would take birds breeding in Ellesmere Island and northern Greenland to Iceland rather than to the Atlantic coast of North America. The likely genetic template for migration is probably to follow a southward bearing, therefore such a shift in direction is feasible given severe orientation complications in regions close to the North Pole where nearly any bearing is south (Gudmundsson & Alerstam 1998). This new migratory route however, requires a hazardous crossing over the Greenland icecap, and Henningsson & Alerstam (2005a) point out that ecological barriers play a large role in shaping migratory routes. Nevertheless, the route from breeding grounds in Ellesmere Island and northern Greenland to staging areas in Iceland is nearly 1000 km shorter than a migration to the Bay of Fundy, and it does not require a full crossing of the Arctic Ocean. Furthermore, this link across the Greenland icecap is used today by other species of shorebirds including Ruddy Turnstones *Arenaria interpres*, Sanderlings *Calidris alba* and Ringed Plovers *Charadrius hiaticula*, indicating that migrant shorebirds are capable of crossing even severe ecological barriers (Henningsson & Alerstam 2005a). Thus, birds utilizing this new route were likely at a selective advantage either in terms of a shorter migration distance (Pienkowski & Evans 1985) or in terms of better staging and wintering areas in Iceland and Europe.
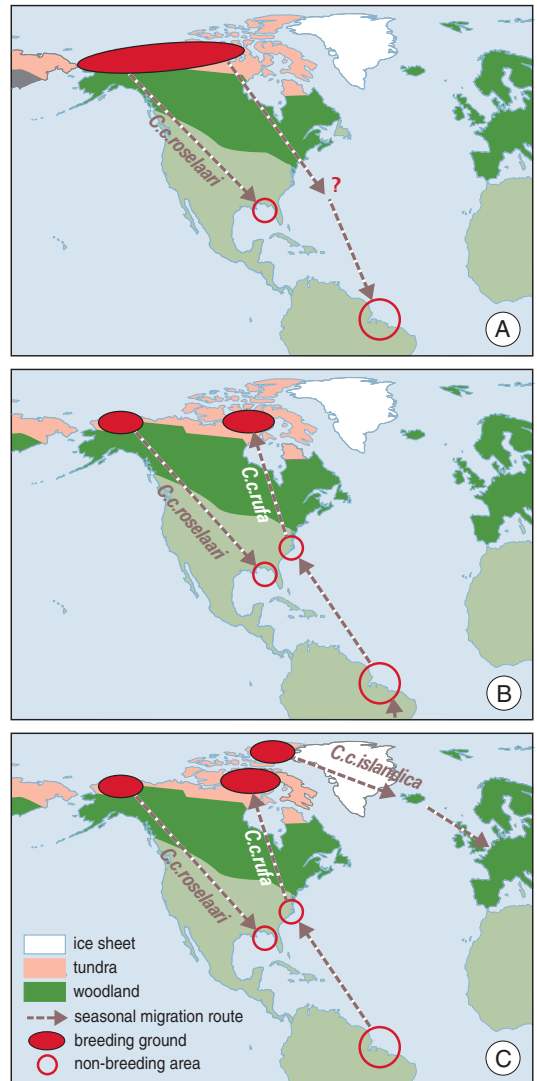

Figure 3. A graphical representation of the proposed range expansions and the establishment of new migratory routes in North America with subsequent expansion into Europe. (A) Possible expansion of knots eastward into the Canadian Arctic Archipelago followed by the establishment of a migratory route into South America. (B) The possible establishment of the contemporary *C. c. rufa* flyway for spring migration after the emergence of Horseshoe crab spawning grounds along the eastern seaboard of the United States. (C) Possible expansion of knots into the High Canadian Arctic and subsequent pioneering of a migratory route via Iceland into Europe.

Favourable staging and wintering areas became available in the Wadden Sea, the British Isles and Iceland 4–5000 years ago (Vos & van Kesteren 2000, Simonarson & Leifsdottir 2002) and would thus have been available to *C. c. islandica* pioneers of a new migratory route (Fig. 3C). Current conditions in Iceland and northwest Europe continue to provide a suitable climate for staging and wintering birds at higher latitudes than the Atlantic coast of North America due to the moderating effect of the North Atlantic current.

CAN THIS HYPOTHESIS BE TESTED?

The palaeovegetation record is not perfect, and our hypotheses are based on population divergence times from a single genetic marker with broad 95% credibility intervals. Nevertheless, these divergence times correlate well with major events during the Pleistocene, events which have had an impact on patterns of species distribution of many other species in the circumpolar Arctic (Henningsson & Alerstam 2005b). Clearly, the testing of this hypothesis of circumpolar expansion eastwards from Asia into North America and then into Europe is needed.

The first question to ask is whether such an expansion has occurred in other species. The circumpolar range expansion of the Herring Gull *Larus argentatus* complex has been the focus of much research as a classic example of the 'ring species model' (Mayr 1942). Recently, work on mitochondrial DNA variation among 21 gull taxa, has shown that the complex differentiated largely in allopatry following multiple segregation and long-distance-colonization events (Liebers *et al.* 2004). Although this finding argues against Mayr's 'ring species model', the results provide an example of an eastward expansion into North America much like that which we argue occurred in Red Knots. According to Liebers *et al.* (2004), two gull taxa (*L. glaucoides* and *L. smithsonianus*) colonized North America across the Bering Strait from an Aralo-Caspian refugium. Unlike in Red Knots,

there is no subsequent development of a migratory route back to Europe. However, gulls are ecologically very different from knots and to the best of our knowledge there are no examples of this type of expansion in shorebirds. This is because very little genetic work has been done on shorebirds, at least at the intraspecific level, with the exception of Red Knots and Dunlins, which have very different demographic histories (Wenink *et al.* 1993, Wennerberg 2001, Buehler & Baker 2005).

An interesting way to test our hypothesis may be to expand upon the Red Knot versus Dunlin comparison presented by Buehler & Baker (2005) and examine population divergence times in shorebirds with circumpolar breeding ranges both in the extreme High-arctic such as the Red Knot and in the Lower-arctic such as the Dunlin. It is possible that the hypotheses of a breeding ground to wintering ground versus a wintering ground to breeding ground development of the Greenland/ Iceland flyway need not be mutually exclusive. Red knots and Dunlins have different ecological requirements, with knots being more restricted to High-arctic breeding areas and marine wintering sites. It is possible that less constrained species such as Dunlins extended their breeding ranges towards Iceland/Greenland by jump dispersal and developed migration along the axis of this range expansion, as supported by the closer genetic relationship between birds using the Greenland/ Iceland flyway to other European lineages than to North American lineages. In contrast, it is possible that High-arctic breeders, especially those with known low genetic variability such as Ruddy Turnstones and Sanderlings (Baker *et al.* 1994), may have expanded their ranges from recently bottlenecked ancestral stock under the same environmental conditions as Red Knots and may thus also have pioneered the Greenland/Iceland flyway from the breeding to the wintering grounds. If this hypothesis is correct for High-arctic breeders, we predict very recent population divergence times between lineages breeding in North America and older divergence dates from Eurasian breeding lineages of these species.

IMPLICATIONS FOR STRICT GENETIC PROGRAMMING OF MIGRATION

There is an element of speculation involved when examining what may have happened to birds pioneering new migratory routes, especially in the distant past. The idea of individuals establishing completely new migratory flyways is controversial. Sutherland (1998) has argued that migratory behaviour is either genetically or culturally determined and that the development of new migratory routes is more likely in species with culturally determined migration. However, the ideas discussed in this paper argue against an absolute genetic program for migratory routes in waders.

We discuss the recent engineering of six different migratory routes within the last glacial of the Pleistocene, at least two of which (those of *C. c. rufa* and *C. c. islandica*) probably occurred within the last few millennia as suitable breeding and wintering areas became available during deglaciation. Range expansions and concurrent development of new migratory routes in arctic-breeding species as ice receded after the last glacial period was not unique to Red Knots and must have occurred all over the northern hemisphere. This recent establishment of migratory routes must have occurred not only in species with culturally determined migration, but also in species like knots, in which juveniles perform their first migration unaccompanied by their parents, thus falling into the category of 'genetically determined migrants'. During periods of climatic upheaval such as the Pleistocene an absolute genetic program would result in birds flying to their deaths as breeding and wintering areas became ice covered or flooded, and we feel that this is unlikely in a whole suite of 'genetically determined migrants'. What may be more likely is a simple genetic template for migratory behaviour, such as a predisposition to follow a southward bearing, accompanied by learned behaviour that responds to the environment to perfect the new migratory route.

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SAMENVATTING

Tijdens de afwisseling van glacialen en interglacialen tijdens het Pleistoceen en in het huidige Holoceen zijn de trekpatronen van vogels dramatisch veranderd. De onderhavige studie formuleert een hypothese omtrent de evolutie van de trekbanen van de Kanoet *Calidris canutus*. Dit gebeurt op grond van (1) moleculaire bepaling van het tijdstip van divergentie tussen populaties en (2) de ontwikkeling van de vegetatie in het verre verleden rond de Noordpool en van getijdengebieden over de gehele wereld. Analyses van mitochondriaal DNA geven aan dat *C. c. canutus* ongeveer 20.0000 jaar (95% betrouwbaarheidsinterval 4000–60.000) geleden divergeerde uit de Meest Recente Gemeenschappelijke Voorouder (MRGV) van de Kanoeten. Ongeveer 12.000 (3500–45.000) jaar geleden divergeerde deze MRGV in twee lijnen, tegenwoordig vertegenwoordigd door de Noord-Amerikaanse populaties *C. c. roselaari, C. c. rufa* en *C. c. islandica* en de Siberische populaties *C. c. piersmai* and *C. c. rogersi*. Het tijdstip van divergentie van de twee Siberische ondersoorten viel ongeveer 6500 (1000–25.000) jaren geleden, en de Noord-Amerikaanse ondersoorten hebben zich pas gedurende de laatste 1000 jaar afgesplitst. Deze tijdstippen van divergentie suggereren dat alle voorouderpopulaties van de Kanoet ontstaan zijn in het laatste glaciaal van het Pleistoceen via een expansie naar het oosten Noord-Amerika in. Dit betekent dat tegen algemeen geldende inzichten in *C. c. islandica* niet ontstaan is uit *C. c. canutus* ook al lijken ze sterk op elkaar en overlappen hun huidige trekroutes in het Waddengebied. In plaats daarvan is *C. c. islandica* het meest verwant met de andere Noord-Amerikaanse ondersoorten *C. c. roselaari* en *C. c. rufa*. Het lijkt erop dat *C. c. islandica* slechts recent de huidige trekroute naar Europa heeft ontwikkeld, en wel nadat de omstandigheden in de winter in het Waddengebied waren verbeterd en er geschikte plekken om te pleisteren op IJsland waren ontstaan. Dit betekent dat – althans voor de Kanoet – de trekroute van Groenland naar IJsland pas zeer recent is ontstaan vanuit de broedgebieden in Amerika naar Europa en niet omgekeerd zoals eerder gedacht.