Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences

D. LIEBERS*† & A. J. HELBIG*

*University of Greifswald, Vogelwarte Hiddensee, Kloster, Germany
†Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

Keywords:

historical demography;
Larus argentatus–fuscus–cachinnans group;
mitochondrial control region;
population genetic structure.

Abstract

Because of the differential amplitude of climatic oscillations, species living at northern latitudes are subject to more frequent and more severe range oscillations than species at southern latitudes. As a consequence, northern populations should, on average, be phylogenetically younger and possess less phylogeographical structure than closely related taxa further south. To test these predictions, we studied the mitochondrial-genetic population structure of NW Palearctic Lesser Black-backed Gulls (*Larus fuscus* group [=LBBG], five taxa) breeding at temperate to boreal latitudes from Iceland to the Taimyr Peninsula. Results were compared with those previously obtained (Liebers *et al*. 2001. *Mol. Ecol.* **10**: 2447) for more southerly breeding Yellow-legged Gulls (*Larus cachinnans* group, six taxa from the Atlantic Islands to Mongolia). Sequences of the hypervariable region I (HVR-I) of the mitochondrial control region revealed low within- and between-taxon sequence divergence, little genetic variation, a shallow haplotype phylogeny and poor phylogeographical structure in LBBGs compared with Yellow-legged Gulls. Haplotype frequencies among the five northern taxa formed a stepped cline with significant gene flow restriction between the forms *heuglini* and *fuscus*, probably indicating a secondary contact with (partial?) reproductive isolation. Western forms of LBBG, among which mitochondrial gene flow appears unrestricted, show genetic signs of postglacial range expansion and population growth. The *Larus fuscus* group is derived from a *cachinnans*-like ancestral population, probably in the Aralo-Caspian basin, and spread from east (NW Siberia) to west within the Palearctic.

Introduction

Long-term climatic oscillations (Milankovitch cycles) force species living at northerly latitudes to retreat from and recolonize their ranges repeatedly over the course of a species' life span (Dynesius & Jansson, 2000; Hewitt, 2000). The influence of quaternary glacial cycles on the genetic population structure is likely to have been much more pronounced in species currently living at boreal and subarctic latitudes than those living further south. Two factors associated with strong range oscillations tend to reduce genetic variability: (1) the drop in population size associated with isolation in refugia and (2) the sequential bottlenecking during subsequent rapid recolonization of northern latitudes (Hewitt, 1996; Ibrahim *et al*., 1996). In addition, northern taxa are selected for high dispersal capacity which should lead to homogenizing gene flow over large areas. If ranges were fragmented into several refugia during glacial maxima, gene flow during subsequent range expansion will lead to mixing of refugial gene pools unless isolation lasted long enough for some reproductive isolation to evolve. In general therefore species at northerly latitudes should show less phylogeographical population structure, and thus be less likely to speciate,

Correspondence: Andreas J. Helbig, Vogelwarte Hiddensee, University of Greifswald, Zum Hochland 17, D-18565 Kloster, Germany.
Tel.: +49 38300 212; fax: +49 38300 50443;
e-mail: helbig@mail.uni-greifswald.de

than closely related species further south (Dynesius & Jansson, 2000).

Here we intend to test these predictions by comparing a northern and southern group of large gulls (genus *Larus*) which form part of the Herring – Lesser Black-backed Gull *Larus argentatus–fuscus–cachinnans* complex. Ever since Mayr (1940, 1963) proposed the ring species model, this group of gulls with a circumpolar distribution in the northern hemisphere has served as a textbook example of geographical differentiation and speciation.

Within the western Palearctic three species are distinguished in the Herring – Lesser Black-backed Gull (LBBG) complex according to current taxonomy (Haffer, 1982; Cramp & Simmons, 1983; Burger & Gochfeld, 1996): (1) the southerly distributed Yellow-legged Gull *Larus cachinnans* (six subspecies); (2) the northerly distributed LBBG *Larus fuscus* (subspecies *graellsii, intermedius, fuscus, heuglini, taimyrensis*); and (3) the Herring Gull *Larus argentatus* (subspecies *argentatus*, *argenteus, omissus*). In a recent mitochondrial-genetic study we found strong phylogeographical structure among the six forms of Yellow-legged Gulls breeding from the Atlantic Islands (*atlantis*), through the Mediterranean (*michahellis*), Black Sea and Aralo-Caspian region (*cachinnans*) to SW Siberia (*barabensis*), Anatolia (*armenicus,* usually separated at species level) and Mongolia (*mongolicus*) (Liebers *et al*., 2001).

Here we focus on the northern equivalent of Yellow-legged Gulls, the LBBG *Larus fuscus*. This species constitutes a chain of five subspecies with contiguous breeding ranges along the NW Palearctic coasts from Iceland and Britain in the west (*graellsii*) throughout the North Sea and Scandinavia (*intermedius, fuscus*), the Barent and Kara Seas (*heuglini*) to the Taimyr Peninsula (*taimyrensis*) in the east (Haffer, 1982; Cramp & Simmons, 1983; Burger & Gochfeld, 1996; cf. Fig. 2).

Conflicting hypotheses about the evolutionary origin of LBBGs have been proposed. Based on the similarity between its westernmost subspecies *graellsii* and the Atlantic Yellow-legged Gull ('*Larus atlantis*'), Dwight (1922) considered LBBGs to be derived from the latter and having spread from the NE Atlantic eastwards to western Siberia. In his model, westernmost populations (*graellsii*) would be evolutionarily the oldest, more easterly population becoming progressively younger. Mayr (1940, 1963), on the other hand, suggested that LBBG are derived from an ancestral population in the Aralo-Caspian region, which first spread northward to the Siberian-Arctic coast and secondarily colonized western Europe. According to this model, the eastern forms (*heuglini, taimyrensis*) should be the oldest with more westerly forms being progressively younger. Thus, the two hypotheses make opposite predictions about the relative evolutionary ages of the taxa within the chain. Population-genetic characteristics should allow us to decide between these two alternatives.

Subspecific taxonomy of LBBGs is based on differences in mantle colour, pattern of primary tips, ecology and behaviour (Stegmann, 1934; Barth, 1968, 1975; Haffer, 1982; Cramp & Simmons, 1983; Burger & Gochfeld, 1996). Pronounced phenotypic differences between some of the taxa have recently led to the proposal, based on a phylogenetic species concept, to split them into three species: *L. graellsii* (incl. *intermedius*), *L. fuscus* and *L. heuglini* (incl. *taimyrensis*) (Sangster *et al*., 1998). Nominate *fuscus* appears particularly distinct from the other forms. It is smaller on average, more delicately built, relatively longer-winged, has a darker (blackish) rather than dark-grey mantle, less white in the tips of the outer primaries, a different moult schedule, is an offshore feeder during the nonbreeding period and a long-distance migrant (Barth, 1975; Bergman, 1982; Strann & Vader, 1992; Jonsson, 1998). Its dramatic recent population decline stands in stark contrast to the spectacular spread and population increase that is well-documented for *intermedius* and *graellsii* during the twentieth century (Glutz & Bauer, 1982; Lloyd *et al*., 1991; Holloway, 1996; Hagemeijer & Blair, 1997). There is also a clear migratory divide between *fuscus*, which migrates long distances southward to tropical East Africa, where it winters both on the coast and large inland lakes, and *intermedius*, which migrates short to medium distances towards south-west, wintering primarily along the European and West African Atlantic coast (Baker, 1980; Haffer, 1982; Kilpi & Saurola, 1984).

So far, no genetic studies have been carried out that would tell us whether the obvious phenotypic differentiation between the various LBBG taxa corresponds to significant amounts of genetic differentiation. We used nucleotide sequences of the hypervariable region I (HVR-I) of the mitochondrial control region to quantify the degree of genetic differentiation between the five taxa. In particular, we wanted to (1) test the prediction that the northern LBBG (*fuscus* group) should contain less phylogeographical structure than the southern Yellow-legged Gull (*cachinnans–michahellis* group) and (2) see what population-genetic characteristics can tell us about the relative ages of the five northern taxa and thus about the direction of colonization of their present ranges.

Materials and methods

Collection of samples and DNA sequencing

Blood or tissue samples of 272 unrelated individuals comprising of five LBBG taxa (*graellsii, intermedius, fuscus, heuglini, taimyrensis*) were collected in 22 breeding colonies (Fig. 2). Individuals of the same taxon from geographically close locations were pooled, resulting in a total of 10 'populations' (Table 1). Voucher specimens and aliquots of all samples investigated in this study have been deposited in the Zoological Museum Greifswald.

Table 1 List of taxa investigated, breeding colonies, coordinates, sample sizes (*n*) and grouping of colonies into populations.

Colony	Coordinates	*n*	Population	Taxon
Iceland	64°09′N, 21°57′W	9	NAT	
Faeroe Islands	62°00′N, 07°00′W	35		
Northern England	53°46′N, 02°42′W	20	UK	*graellsii*
Central England	53°25′N, 02°10′W	6		
Netherlands Rotterdam	51°55′N, 04°28′E	25	EUR	
France, Finistére	48°20′N, 04°00′W	5		
Norway, Vest-Agder	58°20′N, 06°40′E	28	NOR	
Germany, North Sea	54°40′N, 08°20′E	16	GER	*intermedius*
Denmark, Saltholm	55°40′N, 12°45′E	17	DEN	
Finland, Vaasa	63°06′N, 21°36′E	13		
Finland, Tampere	61°30′N, 23°45′E	10	WES	
Finland, Helsinki	60°10′N, 24°48′E	5		
Finland, Kuopio	62°54′N, 27°41′E	4		*fuscus*
Finland, Savonlinna	61°52′N, 28°53′E	11		
Finland, Lake Saimaa	61°15′N, 28°15′E	6	EAS	
Russia, White Sea	66°35′N, 32°45′E	4		
Russia, Finnish Bay	59°40′N, 28°20′E	3		
West Siberia	67°40′N, 44°10′E	10		
Kanin Peninsula	67°20′N, 44°10′E	16	WSI	*heuglini*
Petchora Delta	67°00′N, 52°30′E	3		
Pur District	65°30′N, 77°30′E	3		
Taimyr Peninsula	74°10′N, 86°30′E	23	PJA	*taimyrensis*

Laboratory procedures and particular precautions to ensure mitochondrial origin of the sequences have been described in detail elsewhere (Liebers *et al.*, 2001). Briefly, total DNA was isolated following a standard salting-out protocol (Miller *et al.*, 1988). Using the Expand TW Long-fragment PCR system (Boehringer-Mannheim, Germany), we amplified a 2500–3000 bp fraction of the mitochondrial genome which included the entire control region, the ND6 gene and a part of the 12S rRNA gene. From this, a stretch of 430 nucleotides comprising of HVR-I was sequenced directly with primer HLB. Control amplifications with various different primer combinations from total DNA and CsCl purified mtDNA (14 individuals) yielded identical sequences, confirming their mitochondrial origin (all primer sequences reported in Liebers *et al.*, 2001).

Phylogenetic analysis

To estimate the haplotype phylogeny, pairwise Kimura 2-parameter distances (Kimura, 1980) between all haplotypes were computed in MEGA 2.1 (Kumar *et al.*, 2001). Rate heterogeneity among sites was taken into account by assuming γ-distributed substitution rates; the α-parameter was estimated from the sequence matrix using TREE-PUZZLE 5.0 (Strimmer & von Haeseler, 1996). From the resulting distance matrix a haplotype tree was constructed with the Kitsch algorithm (PHYLIP 3.5c; Felsenstein, 1993). A likelihood ratio test (TREE-PUZZLE) confirmed that the molecular clock hypothesis was not violated, i.e. rates of molecular evolution did not differ significantly between lineages. Support values for internal branches of the haplotype phylogeny were calculated by likelihood mapping using the quartet puzzling algorithm (Strimmer & von Haeseler, 1997) with the HKY substitution model (Hasegawa *et al.*, 1985) and 10 000 quartets per branch (TREE-PUZZLE).

To portray relationships and geographical partitioning among haplotypes within Yellow-legged and LBBGs, uncorrected median-joining networks (Bandelt *et al.*, 1999) were computed using the program NETWORK 3.0 (Röhl, 2000). For reasons of clarity, we included only haplotypes occurring at least twice in the respective sample.

Analysis of population structure

To assess mitochondrial genetic diversity within LBBG taxa, numbers of haplotypes (HT), polymorphic sites (*S*), nucleotide diversity (π) with variance $V(\pi)$ and mean number of pairwise differences (*d*) were calculated using the program ARLEQUIN 2.0 (Schneider *et al.*, 2000). An index of sample saturation (SAT; see Helgason *et al.*, 2000) was calculated for each taxon from the sample size (*n*) and the number of haplotypes (HT). SAT values >1 indicate adequate sampling, i.e. a disproportionate increase in sample size would be needed to recover any additional haplotypes. To assess differences between recent and historical population sizes, we used the 'expansion coefficient' S/d, i.e. the ratio of variable sequence positions (*S*) relative to the mean number of pairwise nucleotide differences (*d*) between haplotypes within a taxon. Large values indicate recent population

expansion, small values characterize populations with relatively constant long-term population sizes (von Haeseler *et al.*, 1996). Tajima's *D* statistics (Tajima, 1989) and Fu's F_s-test (Fu, 1997) were calculated in ARLEQUIN to test for selective neutrality. Furthermore, significantly negative *D*-values can be interpreted as signatures of population expansion (Aris-Brosou & Excoffier, 1996).

Mitochondrial genetic differentiation between populations was assessed by calculating pairwise Φ_{ST} values and testing their significance by 10 000 permutations in the program ARLEQUIN. Slatkin's linearized Φ_{ST} values (Slatkin, 1995) between all pairs of populations were used to construct a dendrogram using the Kitsch algorithm (PHYLIP) which illustrates patterns of hierarchical population structure in the data. Gene flow among populations was estimated as [*Nm*], the number of female migrants per generation (Slatkin, 1995). The association between population pairwise geographical and genetic distances (linearized Φ_{ST} values) was assessed by the nonparametric Mantel test using 1000 permutations (MANTEL 2.0; Liedloff, 1999).

Analyses of molecular variance (AMOVA; Excoffier *et al.*, 1992) were performed using ARLEQUIN to study the proportion of total genetic variation attributable to different hierarchical levels based on the geographical distribution of haplotypes and pairwise distances between them. Several groupings of populations were tested to maximize the among-group component of molecular variance, i.e. to determine the maximum degree of phylogeographical structure present in the data.

Estimation of expansion times

The number of pairwise differences within taxa (the mismatch distribution) was used to date the onset of demographic expansion (Rogers & Harpending, 1992) using a nonlinear least square approach as implemented in ARLEQUIN. The expansion time τ was calculated as $\tau = 2ut$ with $u = \mu k$, where μ is the mutation rate per site and year, and k is the sequence length. The 95% confidence intervals (CI) around the expansion time τ were obtained by parametric bootstrapping (see Excoffier & Schneider, 1999). As the mutation rate μ of HVR-I is not known, we estimated it relative to that of the cytochrome b (cyt b) gene. Pairwise maximum likelihood distances [Tamura-Nei model (Tamura & Nei, 1993) with rate heterogeneity; TREE-PUZZLE] derived from complete cyt b sequences ($n = 1143$ bp) were plotted against those derived from HVR-I using 63 gulls of the *michahellis–cachinnans* group for which both kinds of sequence were available (Liebers *et al.*, unpublished data). This showed that HVR-I sequences had diverged on average 5.3 times faster than cyt b sequences. Thus a cyt b divergence rate of 1.6% per Mio years (Fleischer *et al.*, 1998) translates into a HVR-I divergence rate of 8.48% per Mio years, corresponding to a mutation rate of HVR-I sequences of $\mu = 4.24 \times 10^{-8}$ per site per year.

Results

HVR-I sequence variation

The sequence of a 430-bp segment of HVR-I was determined for 272 individuals representing all five LBBG taxa. Thirty-three sites (7.7%) were found to be polymorphic, 14 (3.3%) of which were parsimony informative (Fig. 1). All inferred substitutions were transitions except a single transversion in *taimyrensis* (HT 25, pos. 096). Substitution rates varied strongly among sites resulting in an α-value of 0.08. We detected a total of 44 haplotypes, of which 17 (38.6%) occurred at least twice within the total sample. Figure 1 shows the frequency of each haplotype per population, the full length sequence of HT 001 was deposited in the EMBL nucleotide data bank (accession no. AJ277127). One individual from Purina, SW Siberia, carried a haplotype typical of Aralo-Caspian 'Steppe Gulls' *L. cachinnans* (HT 212). This was interpreted as recent introgression into the breeding range of *heuglini* (cf. Liebers *et al.*, 2001). In the phylogenetic analysis, HT 212 was used as the outgroup haplotype, but it was excluded from all population genetic analyses.

The index of sample saturation (SAT, Table 2) indicates that *graellsii* and *intermedius* were sampled adequately (values >1), in *heuglini* and *fuscus* sampling was close to adequate, whereas *taimyrensis* ($n = 23$) was clearly undersampled, i.e. more haplotypes (in addition to those found) are expected to exist in the population.

Within-taxon diversity

In all LBBG taxa, two haplotypes (HT 001 and 011) were numerically dominant that differed only by one substitution (Figs 1 & 3). All other 42 haplotypes in our sample were relatively rare; in fact, 32 of them were found in only one population and 26 in a single individual. Indices of within-taxon genetic diversity such as mean sequence divergence (d) and nucleotide diversity (π) increased from west to east, i.e. they were lowest in *graellsii* (Table 2). Westernmost taxa (*graellsii, intermedius*) also showed the highest expansion coefficient S/d (Table 2), consistent with their well-documented population increase during the past century.

Haplotype phylogeny and geographical frequencies

A Kitsch tree of all haplotypes, rooted with the sequence of *Larus cachinnans* (HT 212), revealed two major clades (Fig. 1). Clade I (green portion in pie charts, Fig. 2) was characterized by a C–T transition (relative to all other sequences) at nucleotide position 131, with one haplotype (HT 011) accounting for 43% of the total sample. The other major clade II (light blue in Fig. 2) lacked any unique autapomorphic substitution, thus its monophyly was not significantly supported. Here again, one haplotype was numerically dominant (HT 001 at 24% of

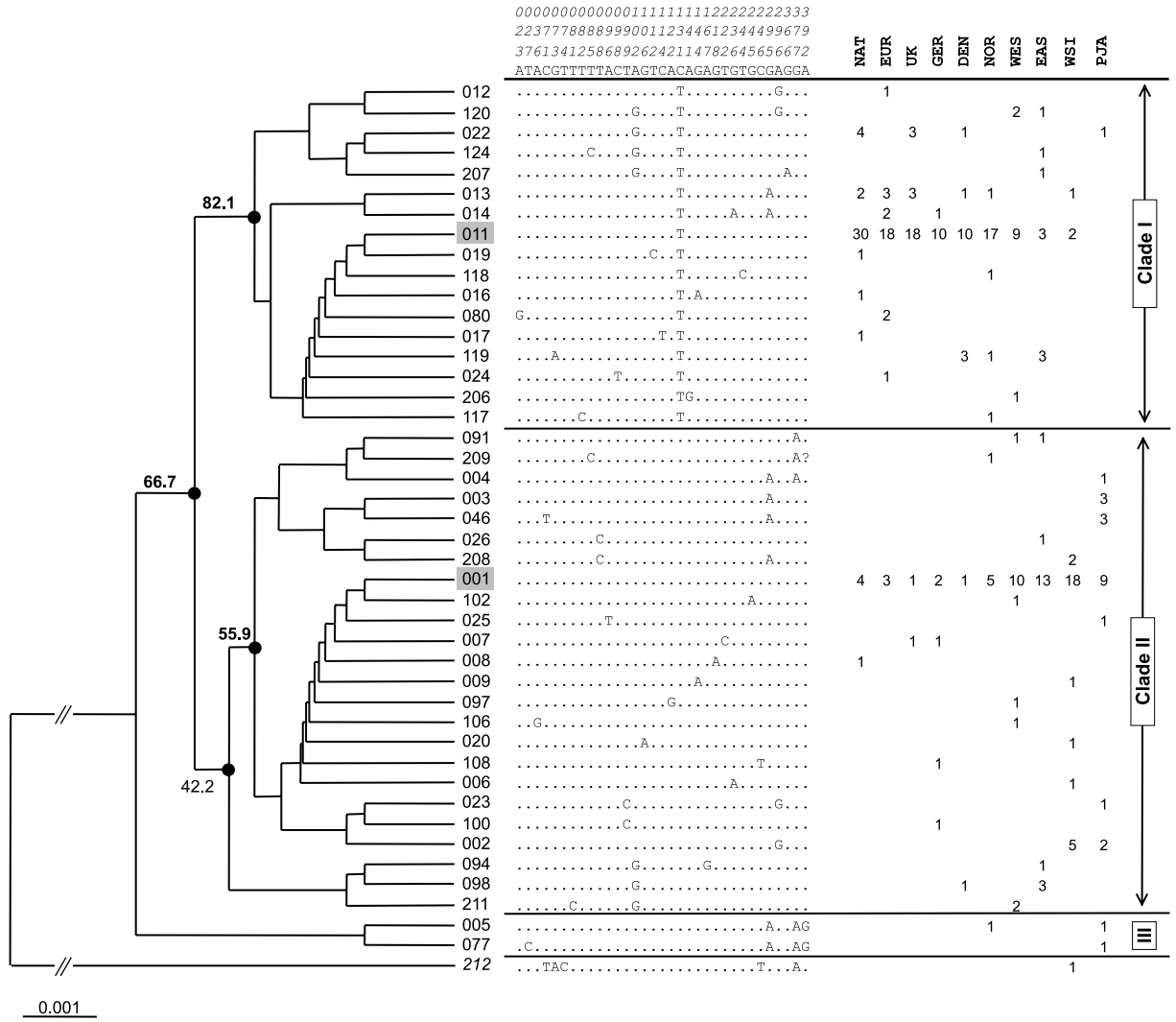

Fig. 1 Haplotype phylogeny, variable site matrix (middle portion) and frequency of haplotypes across populations (abbreviations see Table 1). The Kitsch-tree on the left was constructed from Kimura 2-parameter distances ($n = 44$ sequences, HT 212 typical for southern *cachinnans* was used to root the tree). Support values for internal branches were derived by likelihood mapping (TREE-PUZZLE 5.0; Strimmer & von Haeseler, 1997). The site matrix shows variable positions relative to haplotype 001 (position no. 1 corresponds to position no. 38 in the *Calidris alpina* sequence of Wenink *et al.*, 1994).

Table 2 Diversity parameter for five Lesser Black-backed Gull taxa estimated from mtDNA HVR-1 sequences (ARLEQUIN 2.0; Schneider *et al.*, 2000). Number of individuals (n); number of haplotypes (HT), saturation index (SAT), nucleotide diversity ($\pi \times 10^{-3}$) with variance $V(\pi) \times 10^{-3}$, number of variable sites (S), mean number of pairwise sequence differences (d) and corresponding 'expansion coefficient' (S/d), Tajima's D statistics and expansion times expressed in units of mutation rate ($\tau = 2ut$) and in $t = 1000$ years (KY).

Taxons	*n*	HT	SAT	$\pi \pm V(\pi)$	*S*	*d*	*S*/*d*	*D*	τ	*t* (KY)†	CI (KY)‡
graellsü	100	14	2.50	1.75 + 1.5	13	0.73	17.8	−1.837*	0.800	21.9	6–33
intermedius	61	14	1.02	2.32 + 1.8	13	1.00	13.0	−1.857*	0.978	26.8	11–57
fuscus	56	15	0.80	3.33 + 2.3	14	1.43	9.8	−1.579*	1.532	42.0	11–70
heuglini§	31	8	0.78	2.23 + 1.7	7	0.96	7.3	−1.327	1.043	28.6	0–55
taimyrensis	23	10	0.33	4.21 + 2.8	10	1.81	5.5	−1.118	1.896	52.0	1–96

*$P < 0.05$. †Expansion time t obtained from the estimated τ value assuming a mutation rate of 4.24×10^{-8} per site per year. ‡95% Confidence interval (CI) around expansion time t expressed in KY. §Excluding HT 212 (introgression from *cachinnans*).

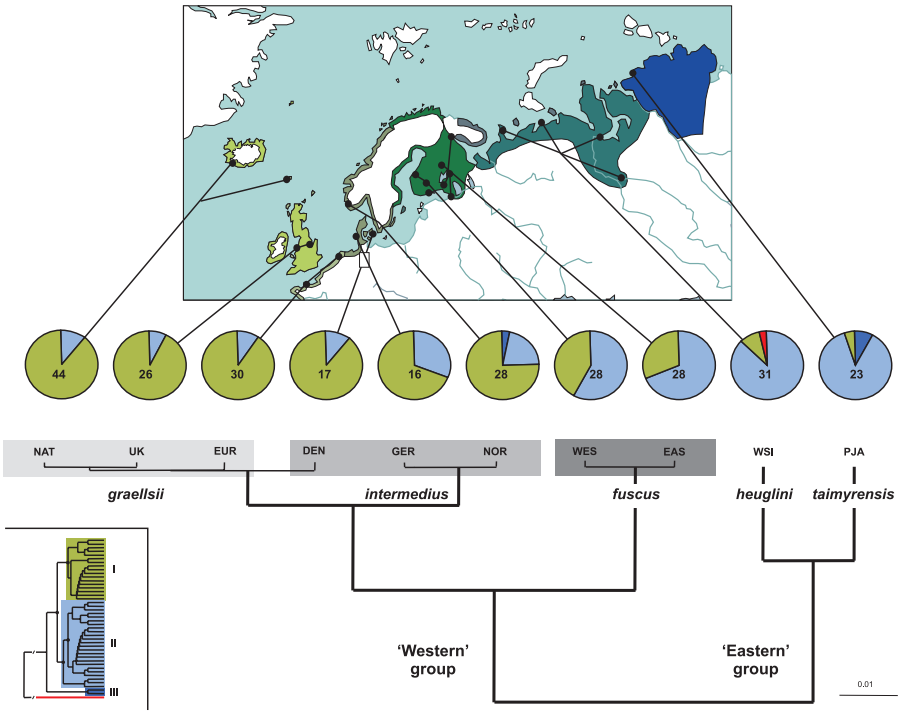

Fig. 2 Haplotype composition (pie charts) of 10 'populations' of Lesser Black-backed Gulls (abbreviations see Table 1). The map shows breeding ranges of the five taxa and sampling locations (coordinates see Table 1). Colours in the pie charts correspond to those in the haplotype phylogeny (see inset; red = *cachinnans* haplotype). The dendrogram below shows the genetic relationships among populations and taxa based on Slatkin's linearized Φ_{ST} values (ARLEQUIN 2.0; Schneider *et al.*, 2000; unrooted Kitsch tree). Branch lengths are proportional to the current level of mitochondrial genetic differentiation.

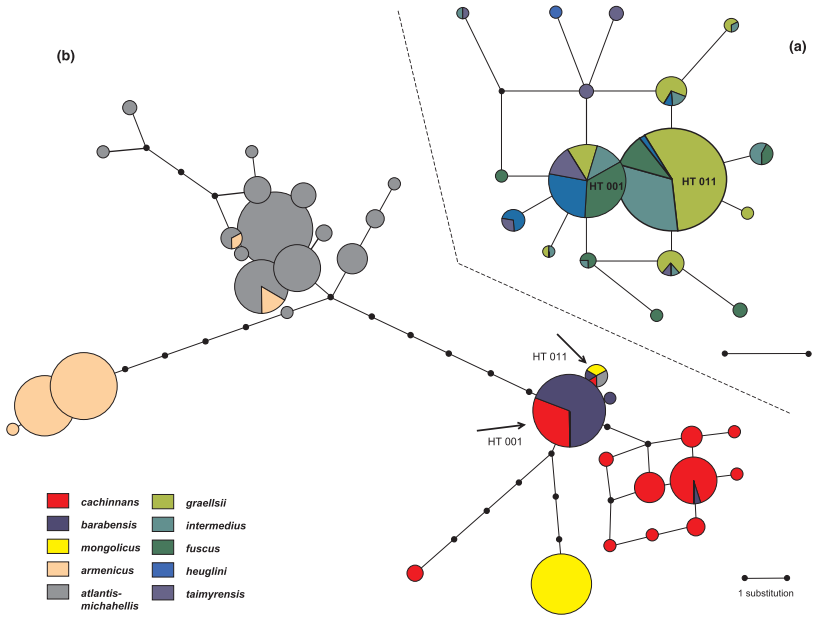

Fig. 3 Median-joining networks (NETWORK 3.0; Röhl, 2000) of mitochondrial control region (HVR-I) haplotypes of (a) five Lesser Black-backed Gull taxa and (b) six Yellow-legged Gull taxa (data from Liebers *et al.*, 2001). Included are only haplotypes that were found at least twice. Size of circles is proportional to frequency, black dots indicate inferred haplotypes. Arrows in (b) highlight haplotypes 001 and 011 which are numerically dominant in (a).

the total sample). A minor, third clade comprised of two haplotypes that are more common in east Siberian and Pacific gull taxa (*vegae*, *schistisagus*; data not shown), suggesting some introgression (mostly into *taimyrensis*) from further east.

Each of the five taxa contained haplotypes of both major clades, albeit at different frequencies. Some segregation of haplotypes between taxa is obvious (see below), but no taxon was exclusively characterized by a particular haplotype (combination). In other words, lineage sorting between taxa was found to be far from complete. However, the relative frequencies of clades I and II haplotypes per population showed an obvious east–west gradient with 'steps' of most pronounced frequency changes between *heuglini* and *fuscus* and between *fuscus* and *intermedius* (Fig. 2).

Population differentiation and gene flow

The 10 populations we sampled were distributed over a distance of 4350 km along a west-east axis (Fig. 2). The level of pairwise population differentiation as measured by Slatkin's linearized Φ_{ST} was strongly correlated with geographical distance (Mantel test: $Z = 60\,059$; $r = 0.774$; $P = 0.002$). This indicates that LBBG populations are not panmictic over large distances. Instead, an isolation-by-distance model, perhaps in addition to weak intrinsic gene flow barriers, more adequately explains the mitochondrial genetic population structure.

The mean sequence divergence between the eastern (*heuglini, taimyrensis*) and western (*fuscus, intermedius, graellsii*) groups of taxa was only 0.23% (= median of one nucleotide difference per 430 bp sequenced), i.e. no larger than the mean sequence divergence within each taxon (except *graellsii*; see *d*-values in Table 2). Thus between-taxon sequence divergence was much lower than for any pairwise comparison among the six taxa of Yellow-legged Gulls, where the mean divergence ranged from 0.46 to 3.48% (Liebers *et al*., 2001).

Population differentiation and gene flow relationships were assessed by pairwise Φ_{ST} values (Table 3) and estimated numbers of female migrants per generation (Fig. 4, Table 3). *Heuglini* and *taimyrensis* were significantly differentiated from populations of all other taxa and, less strongly, among themselves. Nominate *fuscus* was differentiated from all *graellsii* and *intermedius* populations except the one on Amrum, Germany (note small sample size). No differentiation was found between any of the *graellsii* and *intermedius* populations. This pattern of differentiation is consistent with free gene flow between all *graellsii* and *intermedius* populations as well as within the range of nominate *fuscus* (Fig. 4). However, it indicates a significant barrier to mitochondrial gene flow in the contact area between *heuglini* and *fuscus*. Gene flow relations between *fuscus* and *intermedius* are more difficult to interpret, because there is a gap of about 900 km between sampling sites. Sampling closer to areas of possible contact (Swedish east coast, northern Norwegian coast) is needed to resolve this question.

Population expansion

There was evidence for significant population expansion at least in *fuscus*, *intermedius* and *graellsii* (negative Tajima's *D*; $P < 0.05$; Table 2). Fu's F_s-test even rejected population stasis in all five taxa of LBBG ($P < 0.01$), indicating an excess of recent mutations and thus population increase. Crude estimates of when this process began, indicate that ancestral populations of *graellsii* and *intermedius* started expanding 21 900 and 26 800 years ago. Population expansion in *fuscus* and *taimyrensis* started clearly earlier, approximately between 42 000 and 52 000 years ago (but note large confidence intervals; Table 2). This roughly coincides with the deglaciation of the Upper Yenisei River area around 50 000 years BP (Rutter, 1995). The fact that the corresponding value for *heuglini* is lower (28 600 years) is most likely because of a population bottleneck prior to the most recent expansion. Consistent

Table 3 Population pairwise genetic distances (ARLEQUIN 2.0; *settings*: Kimura 2-parameter distance, $\alpha = 0.08$). Above diagonal: inferred number of migrants (*Nm*). Below diagonal: pairwise Φ_{ST} values (bold: $P < 0.01$ with 10 000 permutations).

	NAT	UK	EUR	GER	DEN	NOR	WES	EAS	WSI	PJA
NAT		inf.	21.68	10.56	68.77	18.16	2.25	1.44	0.58	0.58
UK	−0.015		201.64	9.00	247.36	14.05	2.17	1.47	0.57	0.67
EUR	0.023	0.002		13.63	14.15	17.67	2.10	1.38	0.68	0.76
GER	0.045	0.053	0.035		11.27	inf.	10.38	4.01	1.44	1.43
DEN	0.007	0.002	0.034	0.042		23.56	2.93	2.18	0.69	0.81
NOR	0.027	0.034	0.028	−0.018	0.021		5.44	2.86	1.12	1.13
WES	**0.182**	**0.187**	**0.192**	0.046	**0.146**	**0.084**		inf.	4.47	2.44
EAS	**0.257**	**0.254**	**0.266**	**0.111**	**0.186**	**0.149**	−0.001		4.71	2.67
WSI	**0.461**	**0.467**	**0.424**	**0.258**	**0.420**	**0.308**	**0.101**	**0.096**		6.66
PJA	**0.463**	**0.428**	**0.396**	**0.259**	**0.382**	**0.307**	**0.170**	**0.158**	**0.070**	

inf.: Infinite number of migrants.

with this interpretation, *heuglini* also shows the lowest numbers of segregating sites and haplotypes (Table 2).

Phylogeographical structure

The fundamental differences between northern LBBG and southern Yellow-legged Gulls become particularly apparent by contrasting median-joining networks of their respective haplotype assortments (Fig. 3). The mitochondrial gene pool of the LBBGs is completely dominated by two very closely related haplotypes (HT 001 and 011) which differ by a single substitution. In contrast, the gene pool of Yellow-legged Gulls (Fig. 3b) contains at least five haplotype clusters falling into two highly divergent groups (further discussed in Liebers *et al*., 2001). Each of these two basal groups is much more divergent in itself than the entire haplotype assortment present in LBBG.

Among LBBGs, a nested analysis of variance found 79% of the molecular variance within populations, only 21% was accounted for by differences between populations (Table 4, model A). As populations within each taxon were not differentiated, the latter component of variance was almost exclusively the result of differences between taxa ('AG' component in Table 4). Assigning taxa to three groups (*graellsii / intermedius, fuscus, heuglini / taimyrensis*) yielded an among-groups variance component of 27.4% (model B, Table 4). Further splitting up of groups (by separating *heuglini* from *taimyrensis* and *intermedius* from *graellsii*) did not account for any additional among-groups variance (models C, D). Thus, the phylogeographical structure of LBBGs is best represented by recognizing three geographical and taxonomic entities.

In Yellow-legged Gulls, the partitioning of molecular variance was opposite to that in LBBG (Table 4): 82% of the total variance was accounted for by differentiation among five taxa (with *atlantis / michahellis* pooled), only 16% of the variance was within populations.

Discussion

Differentiation of LBBG taxa

Our study of mitochondrial HVR-I sequences revealed that the five dark-mantled, NW Palearctic gull taxa collectively called 'LBBG' are genetically very closely related. The extensive sharing of haplotypes among taxa (= incomplete lineage sorting) can be attributed to ongoing gene flow and / or very recent radiation (Avise, 2000). Although none of the five taxa was diagnosable using mitochondrial haplotypes, there were clear differences in haplotype frequencies between them. Indices of within-taxon genetic diversity showed that the eastern taxa (*taimyrensis, heuglini*) are genetically more diverse and have a longer population history than the western taxa. The latter, particularly *graellsii*, are very uniform genetically and show strong signs of recent population expansion. Thus the evidence indicates a westward expansion of LBBG populations from NW Siberia towards the NE Atlantic.

The genetic uniformity of *graellsii* is particularly interesting when contrasted with the population structure of Atlantic Yellow-legged Gulls (*L. michahellis atlantis*). Both inhabit adjacent and overlapping oceanic regions, but *atlantis* is genetically much more diverse with a high nucleotide diversity and low expansion coefficient (Liebers *et al*., 2001). Clearly, demographic histories of

Table 4 Analysis of molecular variance (ARLEQUIN 2.0; *settings*: Kimura 2-parameter distance, $\alpha = 0.08$) for 10 Lesser Black-backed Gull populations (four models, A–D) compared with Yellow-legged Gulls (Kimura 2-parameter distance, $\alpha = 0.04$; see Liebers *et al*., 2001).

Model	Taxa in groups	Variance component	% Variance
Lesser Black-backed Gulls			
(A) One group: all 10 populations	(1) NAT, UK, EUR, NOR, GER, DEN, WES, EAS, WSI, PJA	AP	21.0
		WP: $\Phi_{ST} = 0.210$	79.0
(B) Three groups	(1) NAT, UK, EUR, NOR, GER, DEN	AG: $\Phi_{CT} = 0.274$	27.4
	(2) WES, EAS	AP: $\Phi_{SC} = 0.022$	1.6
	(2) WSI, PJA	WP: $\Phi_{ST} = 0.290$	71.0
(C) Four groups	(1) NAT, UK, EUR, NOR, GER, DEN	AG: $\Phi_{CT} = 0.281$	28.1
	(2) WES, EAS	AP: $\Phi_{SC} = 0.008$	0.6
	(3) WSI, (4) PJA	WP: $\Phi_{ST} = 0.287$	71.3
(D) Five groups: subspecies boundaries	(1) NAT, UK, EUR, (2) NOR, GER, DEN	AG: $\Phi_{CT} = 0.239$	23.9
	(3) WES, EAS	AP: $\Phi_{SC} = 0.002$	0.1
	(4) WSI, (5) PJA	WP: $\Phi_{ST} = 0.240$	76.0
Yellow-legged Gulls			
Five groups	(1) *atlantis*, *michahellis*, (2) *armenicus*	AG: $\Phi_{CT} = 0.820$	82.1
	(3) *cachinnans*, (4) *barabensis*	AP: $\Phi_{SC} = 0.109$	1.9
	(5) *mongolicus*	WP: $\Phi_{ST} = 0.840$	16.0

Variance components: AG = among groups; AP = among populations within groups; WP = within populations. All Φ values are significant at $P < 0.001$ (10 000 random permutations of sequences among populations).

the two taxa must have been quite different. *Atlantis* is probably directly descended from a large ancestral population that did not experience severe bottlenecks or range restrictions during glaciations, because its marine range in the NE Atlantic was not strongly affected by advancing ice sheets. *Graellsii*, on the other hand, is derived from a more easterly, *fuscus*-like ancestor that would have been subject to more severe population bottlenecking during glaciations and may have lost even more genetic variation during its relatively recent westward expansion.

The haplotype frequencies among LBBG taxa corresponded to a stepped cline (Barton, 1983) with the most pronounced 'step' between *heuglini* and *fuscus*. Estimated numbers of migrants were consistent with free gene flow between all *graellsii* and *intermedius* populations, but indicate a significant barrier to mitochondrial gene flow in the contact area between *heuglini* and *fuscus* along the southern White Sea. This is noteworthy, given that there are no ecological or topographical barriers between the ranges of the two taxa. Intrinsic factors such as different habitat preferences, timing of reproduction (Filchagov *et al*., 1992) or slight differences in mate recognition must be responsible for the maintenance of differentiation between them. Although breeding is locally sympatric (even on some of the same islands), neither mixed pairs nor birds with intermediate characters (potential hybrids) have been observed (Filchagov *et al*., 1992).

Population-genetic structure of northern vs. southern gulls

We documented profound differences in the mitochondrial-genetic population structure between northern LBBG and southern Yellow-legged Gulls, which is in good agreement with predictions derived from biogeographical theory (Dynesius & Jansson, 2000; Hewitt, 2000). Average sequence divergence, differentiation between taxa (Φ_{ST}) and the among-taxon component of molecular variance were all much lower in LBBG than in Yellow-legged Gulls. Estimates of the coalescence time of haplotypes found in LBBG are necessarily rough, not only because of the lack of a reliable rate calibration for the Charadriiform HVR-I, but also because of a large stochastic error associated with the small sequence divergence we found. The maximum divergence among LBBG haplotypes was 1.4% (six nucleotide differences among 430 sites, see Fig. 3). Assuming a rate of 8.48% change per 1 Mio years, the ancestral population of modern LBBG is estimated to have lived approximately 165 000 years ago. This contrasts with an equivalent age estimate of 490 000 years for nominate *Larus cachinnans* in the Aralo-Caspian basin (18 nucleotide differences, see Fig. 4 in Liebers *et al*., 2001). This revised estimate is older than the one we published previously, because we now used a better founded calibration rate (see 'Materials and Methods').

Lesser Black-backed Gulls were characterized by star-like haplotype phylogeny centred on two highly dominating haplotypes, while many rare haplotypes differed only by single substitutions. This pattern is typical of recent population expansion (Slatkin & Hudson, 1991; von Haeseler *et al*., 1996; Forster *et al*., 2001). In contrast, southern Yellow-legged Gulls showed a complex haplotype network with multiple, quite divergent clusters (Fig. 3) corresponding to long periods of multiregional differentiation. Northern gulls therefore are not only phylogenetically younger, but also less differentiated and less structured geographically than their closest relatives at more southerly latitudes. Presumably, range oscillations and associated variation in population size during Quaternary glacial cycles have affected LBBG much more than Yellow-legged Gulls, which enjoyed a larger and more stable long-term population size.

Similar patterns have been found in organisms as diverse as grasshoppers (Cooper *et al*., 1995), Nearctic and Palearctic fish species (Bernatchez & Wilson, 1998), European Crested Newts (Wallis & Arntzen, 1989) and North American woodrats (Hayes & Harrison, 1992), but few studies have addressed these questions in birds so far. Atlantic Common Guillemot (*Uria aalge*), a widespread boreal seabird partly overlapping in range and ecologically comparable with *Larus fuscus*, also showed a star-like haplotype phylogeny and little sequence divergence, i.e. signs of recent population expansion (Moum & Árnason, 2001). Guillemots and Razorbills (*Alca torda*) displayed even less geographical partitioning of haplotype variation than LBBG, so their population-genetic architecture conformed to the pattern expected for species with strong range oscillations caused by glacial cycles. The same is true for those landbird species that have been adequately sampled, e.g. the Greenfinch *Carduelis chloris* (Merilä *et al*., 1997) and several passerine species in North America (overview: Zink, 1996). Most of these studies, with the notable exception of one on a Nearctic migratory warbler (Milá *et al*., 2000), did not compare phylogeographical structure of northern populations directly with that of conspecific (or closely related) southern populations/taxa. It is the direct contrast between closely related, ecologically equivalent forms at different latitudes, as we presented it here for gulls, that illustrates the differences in phylogeographical structure most prominently.

Colonization history

Based on the mitochondrial haplotype network (Fig. 3) we can evaluate the conflicting proposals regarding the origin of the LBBG, either from a *cachinnans*-like ancestor in the Aralo-Caspian basin (Mayr, 1940) or from an *atlantis*-like source population in the NE Atlantic Ocean (Dwight, 1922). LBBG haplotypes are much more similar or identical to those of *cachinnans* ('Steppe Gull') but

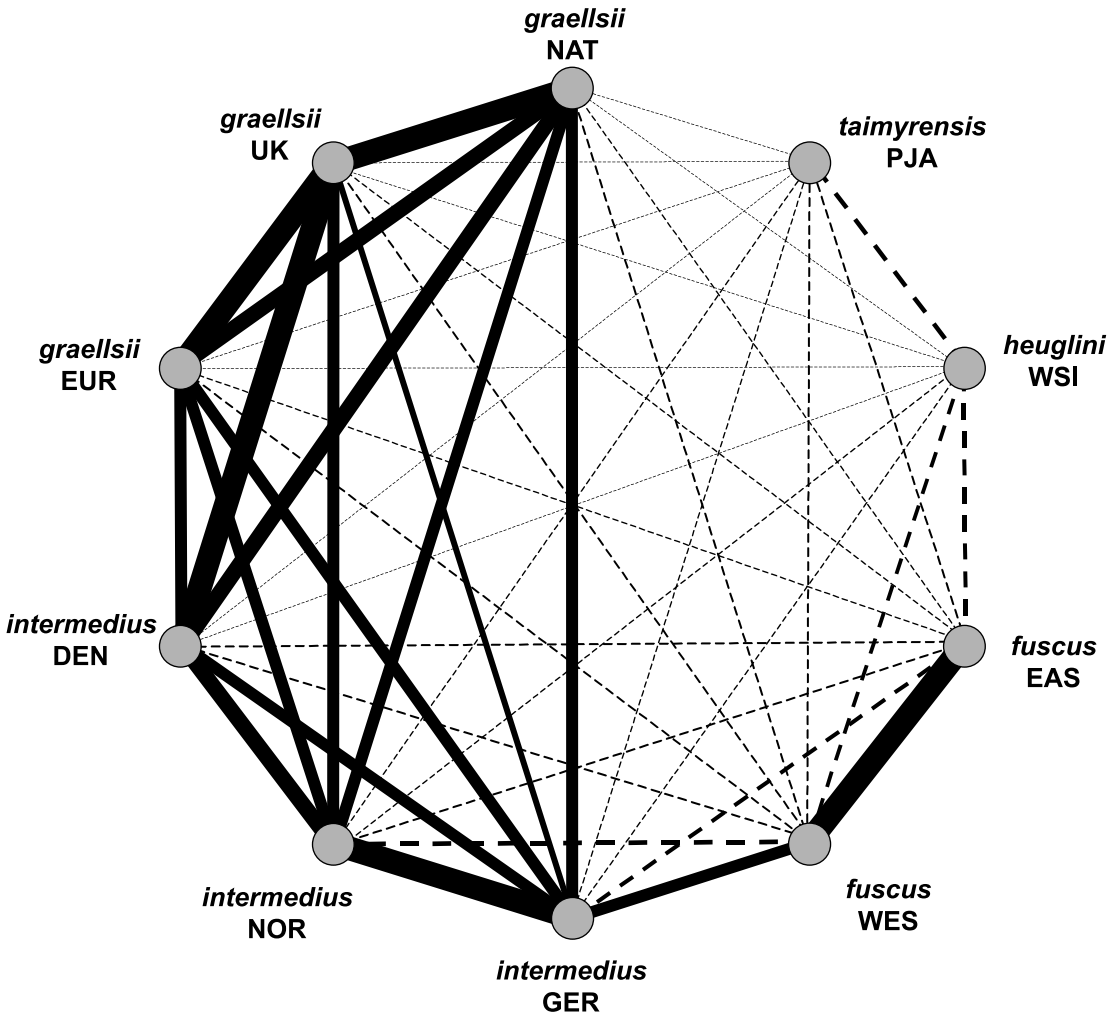

Fig. 4 Model of gene flow relationships among 10 LBBG populations based on *Nm* values (ARLEQUIN 2.0; Schneider *et al.*, 2000). Line thickness is proportional to the estimated number of female migrants per generation. Dashed lines indicate significant population differentiation, based on population pairwise Φ_{ST} values ($P < 0.01$), solid lines correspond to nonsignificant Φ_{ST} values (see Table 3).

highly divergent from *atlantis* (Fig. 3). It is clear therefore that LBBGs are more closely related to Steppe Gull than to Atlantic Yellow-legged Gulls. Our data suggest the following scenario: Ancestors of *Larus fuscus* originated, as Mayr proposed, in the Aralo-Caspian refugium, a vast inland fresh water basin south of the Eurasian ice sheet (Rutter, 1995). During an interglacial they followed the retreating glaciers northward, leading to the foundation of a 'pre-*heuglini*' population in western / central Siberia. Somehow, perhaps during the following glacial maximum, this founder population must have been separated from its ancestral Aralo-Caspian (*cachinnans*-like) population. Possibly, pre-*heuglini* became locked in on ice-free islands in northern Siberia, an area that has been suggested as a refugium of Red-necked Geese (*Branta ruficollis*, Johansen, 1960).

During a later interglacial period gulls from this refugium migrated westward towards ice-free areas in western Siberia and possibly Northern Norway, where they differentiated into precursors of nominate *fuscus*. The contact seen today between *heuglini* and *fuscus* along the White Sea coast must be secondary and relatively recent, following a period of allopatry. A split of the ancestral population into two separate refugia may have occurred during the extensive Weichsel glaciation between 60 000 and 15 000 years BP (compare Dawson, 1992). During much of this period extensive ice sheets formed an effective barrier between ice-free areas in the North Atlantic (possible *fuscus* refugium) and western Siberia with large water bodies in the West Siberian lowlands and the possibly ice-free Kara Sea (potential *heuglini* refugium; cf. Velichko *et al.*, 1984).

Further differentiation into *intermedius* and *graellsii* happened only very recently as indicated by the genetic uniformity, particularly of *graellsii*, and lack of population structure among these two forms. Thus, the paucity of contemporary mtDNA structure may reflect the historical legacy of a rapid and recent westward and southward expansion from Fennoscandia along with considerable population growth.

The taxon *barabensis*, whose current range in the steppes of SW Siberia lies between that of *cachinnans* and *heuglini*, is not differentiated from *heuglini* with respect to mitochondrial haplotypes (Liebers *et al*., 2001). It seems to be a very recent derivative of *heuglini*, although, based on phenotypic characters (pale grey mantle), it has so far been regarded as a subspecies of *L. cachinnans* (but see Panov & Monzikov, 2000). In the light of close relationships between *cachinnans, barabensis* and *heuglini*, the occurrence of a *cachinnans* haplotype in the southern breeding range of *heuglini* (HT 212, Fig. 1) was not surprising. Occasional introgression is expected to occur along with the recent northward range expansion of *cachinnans* into east-central Europe (see Filchagov, 1996; Panov & Monzikov, 1999; Faber *et al*., 2001).

Taxonomic implications

It has been proposed recently (Sangster *et al*., 1998) to divide the NW-Palearctic dark-mantled gulls into three species: LBBG *L. graellsii* (incl. *intermedius*), Baltic Gull *Larus fuscus*, and Tundra Gull *Larus heuglini* (incl. *taimyrensis*). Can this suggestion be justified on the basis of a Biological Species Concept in the light of our mitochondrial-genetic results?

The split between *heuglini* and *fuscus* has received most support from phenotypic differentiation, perceived lack of interbreeding as well as behavioural and ecological segregation (breeding habitat, feeding behaviour) in the area of contact (Stegmann, 1934; Filchagov & Semashko, 1987; Filchagov *et al*., 1992; Rauste, 1999). The significant genetic differentiation we documented certainly indicates a reproductive barrier, although this may be incomplete. More sampling close to the contact zone would be needed to assess more precisely the extent to which gene flow is restricted. Extensive sharing of haplotypes between the two taxa may just reflect the recent separation from a common ancestor (ancestral polymorphism) rather than ongoing gene flow. Based on current evidence, *fuscus* and *heuglini* are best regarded as semispecies (intrinsic gene flow restriction, but probably not complete reproductive isolation).

Evidence for separating *fuscus* from *intermedius*/graellsii is much weaker, both phenotypically and genetically. Notwithstanding the large gap between *fuscus* and *intermedius* sampling sites, the SW Finnish *fuscus* population was only marginally differentiated from *intermedius* in mtDNA. Further sampling in between would probably reveal a smooth cline in haplotype frequencies, compatible with the isolation-by-distance model. Based on phenotypic characters it is also not possible to draw a definite line between *fuscus* and *intermedius* (Jonsson, 1998), because characters vary within each taxon and differ only 'on average' (Barth, 1975; Bergmann, 1982; Cramp & Simmons, 1983; Strann & Vader, 1992). The most clear-cut differences are in moult and migration behaviour, characters that are evolutionarily highly flexible and under strong selection (cf. Helbig, 2002). In conclusion, there is so far no evidence for a significant reproductive barrier between *fuscus* and *intermedius*, they should thus be retained as members of the same species.

Acknowledgments

We thank B. Awadin, A.J. Baker, V.A. Buzun, S.E. Cherenkov, F. Cottaar, B. Ebbinge, A. V. Filchagov, E. Fritze, S. Garthe, M. Hario, T.O. Hansen, J.K. Jensen, R. Juvaste, V.N. Kalyakin, P. de Knijff, P. Mierauskas, K.T. Pedersen, H.-U. Peter, K. Pütz, P. Saurola, A. Sigfusson, R.R. Snell, D. Sowter, P. Stuart, N.v. Swelm, C. Unger, K. Verbeek for their support in acquiring samples for this study, J. Kube for his assistance with Fig. 2 as well as Peter de Knijff and Arndt von Haeseler for help with the analysis and stimulating discussions. This work was supported by grants from the Deutsche Ornithologen-Gesellschaft (DO-G) and Hans-Böckler Stiftung.

References

Aris-Brosou, S. & Excoffier, L. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. *Mol. Biol. Evol.* **13**: 494–504.

Avise, J.C. 2000. *Phylogeography*. Harvard University Press, Cambridge.

Baker, R.R. 1980. The significance of the Lesser Black-backed Gull to models of bird migration. *Bird Study* **27**: 41–50.

Bandelt, H.J., Forster, P. & Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**: 37–48.

Barth, E.K. 1968. The circumpolar systematics of *Larus argentatus* and *Larus fuscus* with special reference to the Norwegian populations. *Nytt. Mag. Zool.* **86**: 1–50.

Barth, E.K. 1975. Taxonomy of *Larus argentatus* and *Larus fuscus* in North-Western Europe. *Ornis Scan.* **6**: 49–63.

Barton, N.H. 1983. Multilocus clines. *Evolution* **37**: 454–471.

Bergman, G. 1982. Population dynamics, colony formation and competition in *Larus argentatus, L. fuscus* and *L. marinus* in the archipelago of Finland. *Ann. Zool. Fenn.* **19**: 143–164.

Bernatchez, L. & Wilson, C.C. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. *Mol. Ecol.* **7**: 431–452.

Burger, J. & Gochfeld, M. 1996. Family Laridae. In: Handbook of the Birds of the World (J. del Hoyo, A. Elliot & J. Sargatal, eds), Vol. III, pp. 572–623. Lynx Edicions, Barcelona.

Cooper, S.J.B., Ibrahim, K.M. & Hewitt, G.M. 1995. Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. *Mol. Ecol.* **4**: 49–60.

Cramp, S. & Simmons, K.E.L. 1983. *The Birds of the Western Palearctic*, Vol. III, pp. 815–837. Oxford University Press, Oxford.

Dawson, A.G. 1992. *Ice Age Earth*. Routledge Press, London.

Dwight, J.M.D. 1922. Description of a new race of the Lesser Black-backed Gull from the Azores. *Am. Mus. Novit.* **44**: 1–2.

Dynesius, M. & Jansson, R. 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proc. Natl. Acad. Sci. USA* **97**: 9115–9120.

Excoffier, L. & Schneider, S. 1999. Why hunter-gatherer populations do not show signs of Pleistocene demographic expansions. *Proc. Natl. Acad. Sci. USA* **96**: 10597–10602.

Excoffier, L., Smouse, P. & Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Faber, M., Betleja, J., Gwiazda, R. & Malczyk, P. 2001. Mixed colonies of large white-headed gulls in southern Poland. *Dutch Birding* **94**: 529–534.

Felsenstein, J. 1993. *Phylip: Phylogeny Inference Package*, Version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA (http://evolution.genetics.washington.edu/phylip.html).

Filchagov, A.V. 1996. Colonization of the central part of the East-European plain by *Larus argentatus-cachinnans-fuscus* gull: geography, parentage of colonists and perspectives. *Ibis* **138**: 148–149.

Filchagov, A.V. & Semashko, V.Y. 1987. Distribution and ecology of the West-Siberian Herring Gull (*Larus argentatus heuglini* Bree 1876) in the Kola peninsula (in russ.). *Biol. Mosk.* **92**: 37–42.

Filchagov, A.V., Bianki, V.V., Cherenkov, A.E. & Semashko, V.Y. 1992. Relations between Lesser Black-backed Gull, *Larus fuscus* and West-Siberian Gull, *L. heuglini*, in the contact zone (in russ.). *Zool. Zh.* **71**: 148–152.

Fleischer, R.C., McIntosh, C.E. & Tarr, C.L. 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Mol. Ecol.* **7**: 533–545.

Forster, P., Torroni, A., Renfrew, C. & Röhl, A. 2001. Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Mol. Biol. Evol.* **18**: 1864–1881.

Fu, Y.X. 1997. Statistical neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915–925.

Glutz von Blotzheim, U.N. & Bauer, K.M. (eds) 1982. *Handbuch der Vögel Mitteleuropas*, Vol. VIII. Akad. Verlagsgesell., Wiesbaden.

von Haeseler, A., Sajantila, A. & Pääbo, S. 1996. The genetical archaeology of the human genome. *Nature Genet.* **14**: 135–140.

Haffer, J. 1982. Systematik und Taxonomie der *Larus argentatus*-Artengruppe. In: Handbuch der Vögel Mitteleuropas (U. N. Glutz von Blotzheim & K. M. Bauer, eds), Vol. VIII, pp. 502–515. Akad. Verlagsgesell., Wiesbaden.

Hagemeijer, W.L.M. & Blair, M.J. 1997. *The EBCC Atlas of European Breeding Birds*. T & AD Poyser, London.

Hasegawa, M., Kishino, H. & Yano, K. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**: 160–174.

Hayes, J.P. & Harrison, R.G. 1992. Variation in mitochondrial DNA and the biogeographic history of Woodrats (*Neotoma*) in the eastern United States. *Syst. Biol.* **41**: 331–344.

Helbig, A.J. 2002. Evolution of bird migration: a phylogenetic and biogeographic perspective. In: *Avian Migration* (P. Berthold & E. Gwinner, eds). Springer, Berlin. In press.

Helgason, A., Sigurdardóttir, S., Gulcher, J.R., Ward, R. & Stefánsson, K. 2000. MtDNA and the origin of the Icelanders: deciphering signals of recent population history. *Am. J. Hum. Genet.* **66**: 999–1016.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**: 247–276.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Holloway, S. 1996. *The Historical Atlas of Breeding Birds in Britain and Ireland*, pp. 1875–1900. Poyser, London.

Ibrahim, K.M., Nichols, R.A. & Hewitt, G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* **77**: 282–291.

Johansen, H. 1960. Die Vogelfauna Westsibiriens. III. Teil. *J. Orn.* **101**: 316–339.

Jonsson, L. 1998. Baltic Lesser Black-backed Gull *Larus fuscus fuscus* – moult, ageing and identification. *Birding World* **11**: 295–317.

Kilpi, M. & Saurola, P. 1984. Migration and wintering strategies of juvenile and adult *Larus marinus*, *L. argentatus* and *L. fuscus*. *Ornis Fenn.* **61**: 1–8.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111–120.

Kumar, S., Tamura, K., Jakobsen, I. & Nei, M. 2001. *Mega: Molecular Evolutionary Genetic Analysis*, Version 2.1. The Pennsylvania State University, University Park, PA 16802 (www.megasoftware.net).

Liebers, D., Helbig, A.J. & de Knijff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves: Charadriiformes). *Mol. Ecol.* **10**: 2447–2462.

Liedloff, A. 1999. *Mantel: Nonparametric Test Calculator*, Version 2.0. Distributed by the author. Queensland University of Technology, Brisbane (http://sci.qut.edu.au/nrs/mantel.htm).

Lloyd, C., Tasker, M.L. & Partridge, K. 1991. *The Status of Seabirds in Britain and Ireland*. Poyser, London.

Mayr, E. 1940. Speciation phenomena in birds. *Am. Nat.* **74**: 249–278.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge.

Merilä, J., Björklund, M. & Baker A.J. 1997. Historical demography and present day population structure of the Greenfinch, *Carduelis chloris* – an analysis of mtDNA control-region sequences. *Evolution* **51**: 946–956.

Milá, B., Girman, D.J., Kimura, M. & Smith, T.B. 2000. Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. *Proc. Royal Soc. Lond. B* **267**: 1033–1040.

Miller, S.A., Dykes, D.O. & Polesky, H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acid Res.* **16**: 1215.

Moum, T. & Árnason, E. 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. *Mol. Ecol.* **10**: 2463–2478.

Panov, E.N. & Monzikov, D.G. 1999. Intergradation between the Herring Gull *Larus argentatus* and the southern Herring Gull *Larus cachinnans*. European Russia (in russ.). *Zool. Zh.* **3**: 334–348.

Panov, E.N. & Monzikov, D.G. 2000. Status of the form *barabensis* within the '*Larus argentatus-cachinnans-fuscus* complex'. *Br. Birds* **93**: 227–241.

Rauste, V. 1999. Kennzeichen und Mauser von 'Baltischen Heringsmöwen' *Larus [fuscus] fuscus* und 'Tundramöwen' *L.* [*fuscus*] *heuglini*. *Limicola* **13**: 105–128, 153–188.

Rogers, A.R. & Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* **9**: 552–568.

Röhl, A. 2000. *Network: A Program Package for Calculating Phylogenetic Networks*, Version 3.0. Mathematisches Seminar, University of Hamburg, Hamburg (www.fluxus-engineering.com).

Rutter, N. 1995. Problematic ice sheets. *Quart. Int.* **28**: 19–37.

Sangster, G., Hazevoet, C.J., Berg, A. & van den & Roselaar, C.S. 1998. Dutch avifaunal list: species concepts, taxonomic instability, and taxonomic changes in 1998. *Dutch Birding* **20**: 22–32.

Schneider, S., Roessli, D. & Excoffier, L. 2000. *Arlequin: A Software for Population Genetic Data. Analysis*, Version 2.0. University of Geneva, Switzerland (http://anthro.unige.ch/arlequin).

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–462.

Slatkin, M. & Hudson, R.R. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**: 555–562.

Stegmann, B. 1934. Ueber die Formen der großen Möwen ('subgenus *Larus*') und ihre gegenseitigen Beziehungen. *J. Orn.* **82**: 340–380.

Strann, K.-B. & Vader, W. 1992. The nominate Lesser Black-backed Gull *Larus fuscus fuscus*, a gull with a tern-like feeding biology, and its recent decrease in Northern Norway. *Ardea* **80**: 133–142.

Strimmer, K. & von Haeseler, A. 1996. Quartet puzzling: a quartet puzzling maximum-likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* **13**: 964–969 (www.tree–puzzle.de).

Strimmer, K. & von Haeseler, A. 1997. Likelihood mapping: a simple method to visualize phylogenetic content of a sequence alignment. *Proc. Natl. Acad. Sci. USA* **94**: 6815–6819.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Tamura, K. & Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**: 512–526.

Velichko, A.A., Isayeva, L.L., Makeyev, V.M., Matishov. G.G. & Faustova, M.A. 1984. Late Pleistocene glaciation of the Arctic Shelf, and the reconstruction of Eurasian ice sheets. In: *Late Quaternary Environments of the Soviet Union* (A. A. Velichko, ed.), pp. 35–44. Longman Group Ltd., London.

Wallis, G.P. & Arntzen, J.W. 1989. Mitochondrial-DNA variation in the Crested Newt superspecies – limited cytoplasmatic gene flow among species. *Evolution* **43**: 88–104.

Wenink, P.W., Baker, A.J. & Tilanus, M.G.J. 1994. Mitochondrial control-region sequences in two shorebirds species, the turnstone and the dunlin, and their utility in population genetic studies. *Mol. Biol. Evol.* **11**: 22–31.

Zink, R.M. 1996. Comparative phylogeography in North American birds. *Evolution* **50**: 308–317.

Received 30 January 2002; revised 6 May 2002; accepted 7 June 2002