The herring gull complex is not a ring species

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Under what circumstances speciation in sexually reproducing animals can occur without geographical disjunction is still controversial. According to the ring-species model, a reproductive barrier may arise through 'isolation by distance' when peripheral populations of a species meet after expanding around some uninhabitable barrier. The classical example of this kind of speciation is the herring gull (*Larus argentatus*) complex, with a circumpolar distribution in the Northern Hemisphere. Based on mitochondrial DNA variation among 21 gull taxa, we show that members of this complex differentiated largely in allopatry following multiple vicariance and long-distance-colonization events, not primarily through isolation by distance. Reproductive isolation evolved more rapidly between some lineages than between others, irrespective of their genetic distance. Extant taxa are the result of divergent as well as reticulate evolution between two ancestral lineages originally separated in a North Atlantic refugium and a continental Eurasian refugium, respectively. Continental birds expanded along the entire north Eurasian coast and via Beringia into North America. Contrary to the ring-species model, we find no genetic evidence for a closure of the circumpolar ring through colonization of Europe by North American herring gulls. However, closure of the ring in the opposite direction may be imminent, with lesser black-backed gulls about to colonize North America.

Keywords: speciation; ring-species model; *Larus argentatus* complex; mitochondrial control region; cytochrome *b* gene; phylogeography

1. INTRODUCTION

Speciation, i.e. the divergence of an ancestral population into two reproductively isolated daughter populations (Dobzhansky 1937), requires genetic differentiation at least at those loci involved in reproductive (sexual) functions. Ongoing gene flow will oppose differentiation between populations, and it is unclear to what extent gene flow must be reduced for speciation to be completed (Slatkin 1987; Turelli *et al.* 2001). Ernst Mayr (1942), based on the earlier ideas of Geyr von Schweppenburg (1938), proposed that reproductive isolation may evolve in a single species through 'isolation by distance', i.e. without interruption of gene flow, when peripheral populations meet after expanding around a large uninhabitable area. This mode of speciation through 'circular overlap' (Mayr 1942) was later termed the 'ring species' model (Cain 1954). Geographical overlap between taxa that are elsewhere connected through interbreeding populations is an essential element of this model, because it is ongoing gene flow that distinguishes ring species from cases of allopatric speciation that happen to be arranged in a roughly circular fashion (Irwin *et al.* 2001*a*).

The classical example that the ring-species model was based upon is the herring gull (*Larus argentatus*) complex. This group comprises more than 20 taxa of large gulls (Haffer 1982), which together occupy a circumpolar breeding range in the Northern Hemisphere. The taxa differ most obviously in body size and in the darkness of their dorsal plumage ('mantle'), which varies from pale grey to black. According to the model of Mayr (1942), the group originated in the Aralo-Caspian region, from where gulls spread in three directions (figure 1*a*):

  (i) west via the Mediterranean into the Atlantic giving rise to Mediterranean (*michahellis*) and Atlantic (*atlantis*) yellow-legged gulls;
 (ii) east towards Inner Asia giving rise to the Mongolian gull (*mongolicus*); and
(iii) north to the Arctic Ocean.

Along the North Eurasian coasts, the ancestral population expanded both ways:

  (i) west across Scandinavia towards Britain and Iceland differentiating into dark-mantled lesser black-backed gulls (*fuscus*, *intermedius* and *graellsii*); and
 (ii) east all the way to the North Pacific, giving rise to the progressively paler-mantled forms *taimyrensis* (Taimyr), *birulai* and *vegae* (eastern Siberia), and into North America (*smithsonianus*).

Following the Last Glacial Maximum, North American herring gulls are supposed to have crossed the North Atlantic and invaded Europe, where they gave rise to the taxon *argentatus* and *argenteus*, which now overlap with lesser black-backed gulls (Geyr von Schweppenburg 1938; Mayr 1942). Mayr envisioned all taxa of the circumpolar chain to be connected by gene flow, while herring gulls and lesser black-backed gulls in Europe, the hypothetical endpoints of the ring, have reached full reproductive isolation and now coexist as distinct species.

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If this scenario is true, we would expect to find (i) extensive sharing of mitochondrial haplotypes resulting from gene flow between geographically neighbouring taxa within the circumpolar ring; and (ii) evidence of introgression or closely related haplotypes between North American (*smithsonianus*) and European (*argentatus*) herring gulls caused by recent transatlantic colonization. We tested this hypothesis by investigating the phylogeography of 20 Northern Hemisphere gull taxa and one Southern Hemisphere gull taxon using 1.5 kb of mitochondrial DNA (mtDNA) sequence.

Previous attempts to test the ring-species model in these gulls were inconclusive owing to the low amount of variation recovered from allozymes (Snell 1991) and short conservative segments of mitochondrial DNA (Crochet *et al.* 2002). The latter study did show that 'herring gull' haplotypes differ between North America (*smithsonianus*) and Europe (*argentatus*), but sample sizes and sequence variation were insufficient to reject the ring-species model. The hypervariable part of the mitochondrial control region (HVR-I), which has been used extensively to document recent evolutionary differentiation of various organisms including humans (Vigilant *et al.* 1991; Baker & Marshall 1997), proved highly informative in gulls (Liebers *et al.* 2001; Liebers & Helbig 2002). Here, we use the HVR-I segment plus the entire cytochrome *b* (cyt *b*) gene in the most comprehensive genetic study of the herring gull complex to date.

2. MATERIAL AND METHODS

(a) *Sampling and taxon designation*

Blood or tissue samples were collected from 410 adults and chicks (unrelated individuals) at breeding sites only (figure 2). Details of sampling locations and sample sizes are given in electronic Appendix A. Taxon designations were based on the phenotype of breeding adults and on geographical location. Voucher material has been deposited at the Zoological Museum Greifswald. Throughout this paper the name '*L. fuscus*' (lesser black-backed gull) comprises the subspecies *L.f. fuscus*, *L.f. intermedius* and *L.f. graellsii*, while '*L. argentatus*' (European herring gull) comprises the subspecies *L.a. argentatus*, *L.a. argenteus* and *L.a. omissus*. The respective subspecies grade into each other so that no clear boundaries can be defined between them. Other taxa that earlier authors considered subspecies of the herring gull (*michahellis*, *smithsonianus*, *vegae*, etc.) are identified by their terminal taxon names, because they are clearly separable phenotypically and/or geographically.

(b) *Laboratory methods*

The cyt *b* gene (1143 bp) was amplified and sequenced on an ABI3700 sequencer using the BigDye terminator cycle sequence kit (Applied Biosystems). Amplification and sequencing were performed in two overlapping fragments using the hybrid primers (5′ → 3′) 1F (L14899)21M13-AAAYAAACYAAT GGCCC + 1R (H15520) M13REV-CAGTTTGATTGAATA CCCAG and 2F (L15488) 21M13-TTACCTTCATCCACC TAAC + 2R (H16061) M13REV-GGTTTACAAGACCA ATG, respectively (H = heavy strand; L = light strand; numbers give the position of the 3′ end in the *Gallus gallus* mitochondrial genome; see Desjardins & Morais 1990). M13REV and 21M13 refer to the universal M13 primers used for sequencing. Fragments were sequenced in both orientations. The mitochondrial

control region was amplified in a 2.5 kb fraction using the Expand Long Template PCR system (Boehringer Mannheim). From this product, which included the entire d-loop, the ND6 gene and a part of the 12S rRNA gene, 430 nucleotides of HVR-I were sequenced directly. Primer sequences and precautions to avoid (co)amplification of possible nuclear copies of mtDNA are detailed in Liebers *et al.* (2001).

(c) *Analysis*
(i) *Alignment*

Cyt *b* sequences aligned without gaps and contained no unexpected stop codons. Alignment of HVR-I sequences required the insertion of single nucleotide gaps at two sites. These two sites were deleted in all sequences prior to further analysis. Sequences were deposited in the EMBL nucleotide sequence data bank (accession numbers cyt *b*: AJ508091–AJ508148; HVR-I: AJ276938–AJ276952, AJ277127–AJ277134, AJ507740–AJ507765, AJ507807–AJ507824 and AJ508304–AJ508346). The full concatenated alignment is also available from the authors. A matrix consisting only of the 116 polymorphic sites was used as input for constructing a median-joining network (Bandelt *et al.* 1999) with NETWORK v. 3111 (www.fluxus-engineering.com/sharenet.htm).

(ii) *Nested-clade analysis*

To estimate relative mutation rates per site, a first median-joining network was constructed by assuming equal weights for each variable position (epsilon set to 0). On the basis of this analysis, recurrent sites were weighted with the reciprocal of their inferred mutation rate, and a second median-joining network (shown in figure 3) was constructed with weights ranging from 0 to 15. All variable positions in cyt *b* and 23 positions in HVR-I received the maximum weight of 15. The single most highly recurrent site received a weight of 0. Using the program TCS v. 1.13 (http://zoology.byu.edu/crandall_lab/tcs.htm) an almost identical network was obtained, which served as the basis for defining a nested-clade design following the guidelines in GEODIS v. 2.0 (http://zoology.byu.edu/crandall_lab/geodis. htm). This clade design was used to perform a nested-clade analysis (NCA) (Templeton 1998) using GEODIS v. 2.0. A number of reticulations in the network, all caused by recurrent d-loop mutations, had to be resolved. For this we performed a number of reduced NCAs comparing only results within subsections of the total network. For instance, for haplogroup A1, we tested six different nested-clade designs, each with a slightly different removal of reticulations. No major differences were detected in NCA results between these slightly different models. Hence, we chose, for each reticulation, one solution by avoiding connections via empty nodes, giving preference to haplotypes more frequent in the sample and more central in the network, and giving priority to connections of haplotypes observed at the same or neighbouring locations.

NCA enables phylogeographical analysis without invoking *a priori* hypotheses. It identifies non-recurring population-history events (population fragmentation, long-distance colonization, contiguous range expansion) and recurrent population-structure events (recurrent gene flow restricted by isolation-by-distance; long-distance dispersal). This is achieved by contrasting the geographical distributions of haplotypes and clades of haplotypes against an evolutionary tree of the same haplotypes. First, the null hypothesis of no association between geography and the haplotype tree was tested. This hypothesis was rejected

($p < 0.05$), so it was justified to interpret the observed pattern in terms of phylogeographical history.

(iii) *Bayesian phylogenetics*

Branch support for the basal structure of the mitochondrial network was estimated using Bayesian phylogenetics (http://morphbank.ebc.uu.se/mrbayes/) (Huelsenbeck *et al.* 2001). From the 160 concatenated haplotypes, four independent consensus trees were constructed using the general time-reversible model with gamma-distributed rate variation. A Markov chain of 1 000 000 generations was simulated. Trees were sampled every 100 generations; the first 200 000 generations were discarded as burn-in. The resulting consensus tree was thus based on 8000 sampled trees. A *Larus occidentalis* haplotype (no. 102, electronic Appendix B) was used to root the tree. Each of the four separate analyses converged on the same topology and log-likelihood and resulted in near-identical posterior branch probabilities. Major clades of this topology were identical to those in the median-joining network. We therefore present posterior probabilities with the network (figure 3).

(iv) *Analysis of molecular variance and dating*

Analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was conducted on all 410 individual sequences with ARLE-QUIN v. 2.0 (http://lgb.unige.ch/arlequin). To date the split between the two major clades (figure 3), we used a coalescence-based method that takes into account possible unequal population sizes and bottleneck effects after the split (Gaggiotti & Excoffier 2000). Only cyt *b* sequences were used to date the split, because a more reliable phylogenetic rate calibration is available for this gene than for HVR-I. Tau (τ) was estimated at 5.631 for the entire cyt *b* haplotype sample excluding the outgroup ($n = 404$). To convert this to an age estimate we assumed a divergence rate of 1.6% per million years (Fleischer *et al.* 1998).

3. RESULTS

The concatenated mitochondrial sequences (1.57 kb) of 410 individuals contained 116 polymorphic sites (70 in cyt *b*, 46 in HVR-I), which defined a total of 160 unique haplotypes (see electronic Appendix B). The haplotype network shows extensive genetic divergence within the herring gull complex (figure 3). The AMOVA indicated strong segregation of haplotypes along taxonomic, i.e. phenotypically defined, boundaries. The taxonomic affiliation of individuals accounted for 54% of the molecular variance. This variance component increased to 71% when geographical structuring within *L. argentatus* and *L. hyperboreus* was taken into account (table 1). The significant taxonomic and geographical structure enables us to interpret the evolutionary history of these gulls based on mitochondrial genetic variation.

The western gull (*Larus occidentalis*) of the North American west coast (range not shown in figure 2) was found to have highly divergent haplotypes relative to all other taxa in this study and is regarded as the outgroup. NCA indicated that the ancestral ingroup population was divided by an allopatric fragmentation event, leading to the evolution of two major clades (figures 3 and 4, see also electronic Appendix D): clade I (haplogroups A–C) centred in the North Atlantic and clade II (haplogroups D–H) with a circumpolar distribution. Assuming a cyt *b*

divergence rate of 1.6% per million years (Fleischer *et al.* 1998), the initial vicariance event occurred some 308 000 (95% CI: 10 200–602 000) years ago. *Larus argentatus* and *L. cachinnans* are the two taxa currently containing the most highly divergent and the earliest-branching haplotype lineages (figure 3). This indicates that they are direct descendants of the two ancestral populations. If current breeding ranges are any indication, ancestors of clade I probably lived in the northeastern Atlantic (current range of *argentatus sensu stricto*), and those ancestral to clade II lived in the Aralo-Caspian region (current range of *cachinnans*; figure 1*b*).

As indicated by NCA, the Aralo-Caspian population (ancestors of clade II) spread by contiguous range expansion towards the north Eurasian coast, then west up to Britain and Iceland (*fuscus* range) and east throughout northern Siberia (*vegae, schistisagus*) and North America (*smithsonianus, glaucescens, glaucoides*; figure 1*b*). In accordance with Mayr's theory, the sharing of haplotypes between adjacent taxa in this circumpolar range indicates ongoing gene flow. However, we find no support for the key element of the ring-species hypothesis, i.e. a transatlantic invasion of North American herring gulls (*smithsonianus*) into Europe. No haplotypes typical of, or derived from, nearctic *smithsonianus* were found anywhere in the European *argentatus* population, not even in Iceland. The endpoints of the circumpolar ring of interbreeding taxa, therefore, do not overlap. Furthermore, yellow-legged gulls of the Atlantic islands (*atlantis*), the Mediterranean Sea (*michahellis*) and Asia Minor (*armenicus*) are derived from North Atlantic (clade I), not Aralo-Caspian ancestors, while central Asia (*mongolicus* range) was colonized from the Pacific coast, not from the Aralo-Caspian basin (*contra* Mayr 1942). The demographic histories of the two basal lineages were quite different according to the respective mismatch distributions (figure 5; for interpretation cf. Avise 2000): among descendants of the North Atlantic refugium (clade I), strong bimodality indicates population growth after long periods of allopatric divergence, whereas descendants of the Aralo-Caspian refugium (clade II) experienced more recent contiguous population expansion.

Two more aspects of the mitochondrial phylogeny are surprising. First, three distinct species previously thought to derive from phylogenetically older ancestors are nested within the herring gull complex: the greater black-backed gull (*L. marinus*) and the glaucous gull (*L. hyperboreus*), which overlap extensively in breeding range with each other and with other members of the assemblage, and the Southern Hemisphere kelp gull (*L. dominicanus*). The latter evolved via long-distance colonization from the same ancestral population as the lesser black-backed gull (figure 4), suggesting that its ancestors were highly migratory, as nominate lesser black-backed gulls still are today.

Second, two taxa are polyphyletic in the mtDNA network: populations of European *argentatus* (pale blue in figures 2 and 3) and of *hyperboreus* (dark grey) each contain haplotypes of both major clades. This can be explained by retention of ancestral polymorphisms and/or by mitochondrial gene flow that occurred after the initial split into two separate refugia. In the case of *argentatus*, where clade I and clade II haplotypes co-occur in the same populations, there is evidence that both processes were
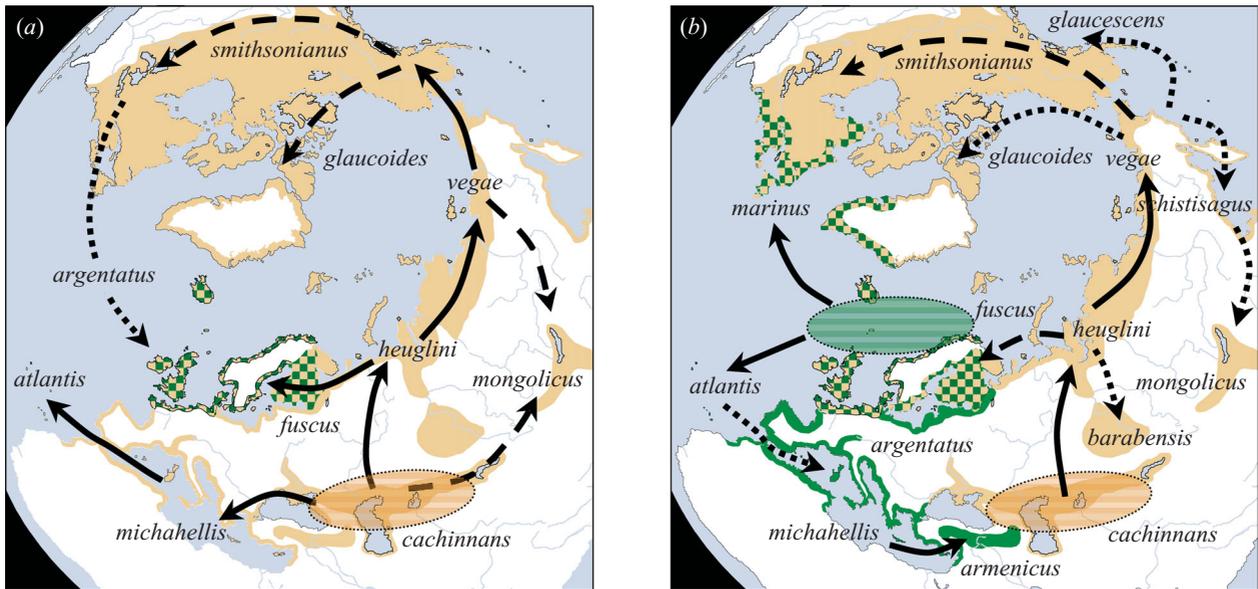
Figure 1. Two hypotheses about the differentiation and colonization history of the herring gull complex. Large ovals show hypothetical refugia. Arrows indicate inferred colonization routes, with temporal progression from ancient to most recent events indicated by solid, broken and stippled arrows. (*a*) The ring-species model of Mayr assumes a single Aralo-Caspian refugium and a most recent invasion of herring gulls from North America to Europe, where they now overlap with lesser black-backed gulls (checkerboard pattern). Mayr did not regard greater black-backed gulls (*Larus marinus*) as part of this complex. (*b*) Alternative model based on the results of the present study. Two ancient refugia are inferred. Current ranges of taxa derived from the Atlantic refugium are shown in green; those derived from the Aralo-Caspian refugium are shown in pale ochre; checkerboard pattern shows areas of overlap. No invasion of herring gulls from North America to Europe occurred. *Larus marinus* developed reproductive isolation in allopatry (probably in northeastern North America) before making secondary contact with North American *smithsonianus* and Eurasian *argentatus* and *fuscus*. Two separate colonization events from the Atlantic into the Mediterranean led to the differentiation of *armenicus* and, much later, *michahellis*.
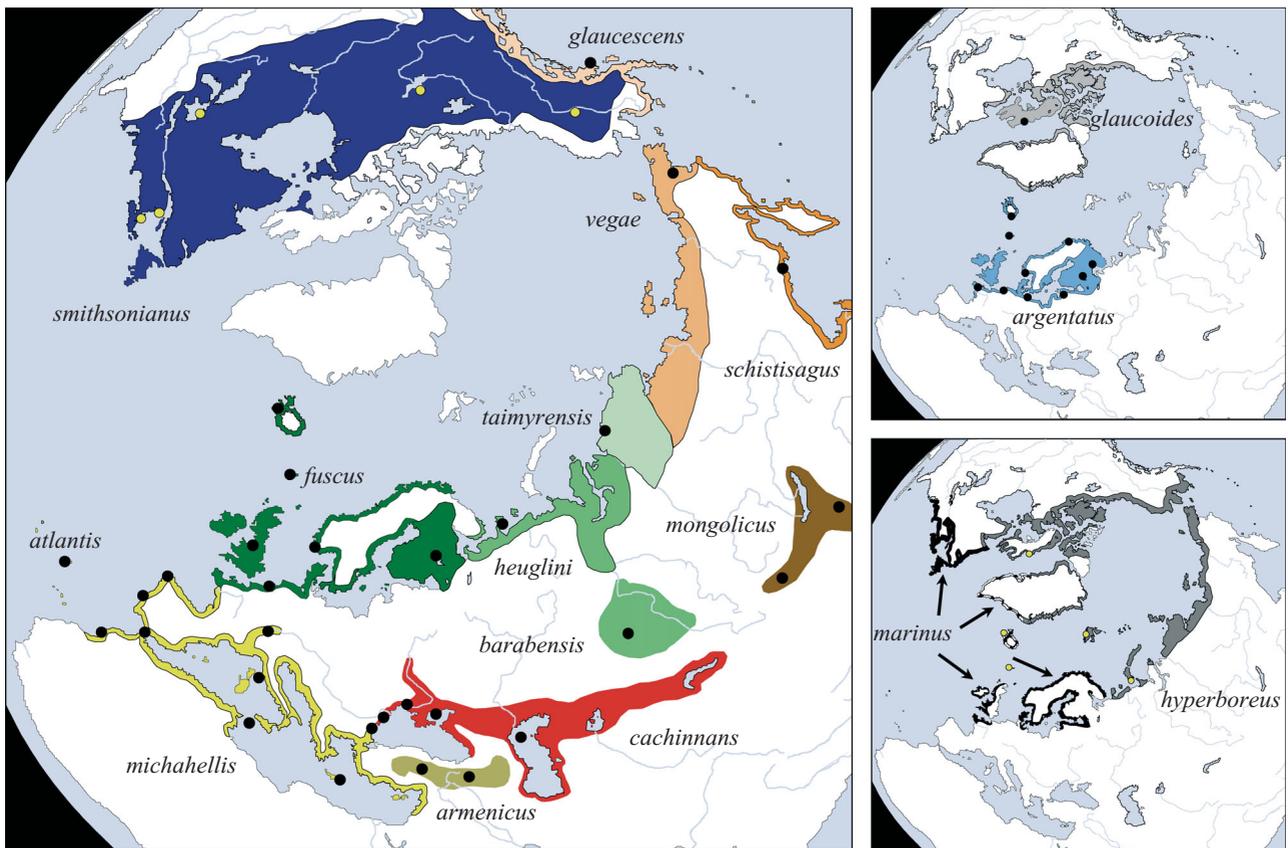

Figure 2. Breeding ranges and sampling locations (dots) of the gull taxa investigated (see electronic Appendix A for details). For reasons of clarity, extensively overlapping ranges are shown on separate maps. Ranges of *Larus occidentalis* and *L. dominicanus* are not shown. See figure 3 for a key to the colour.
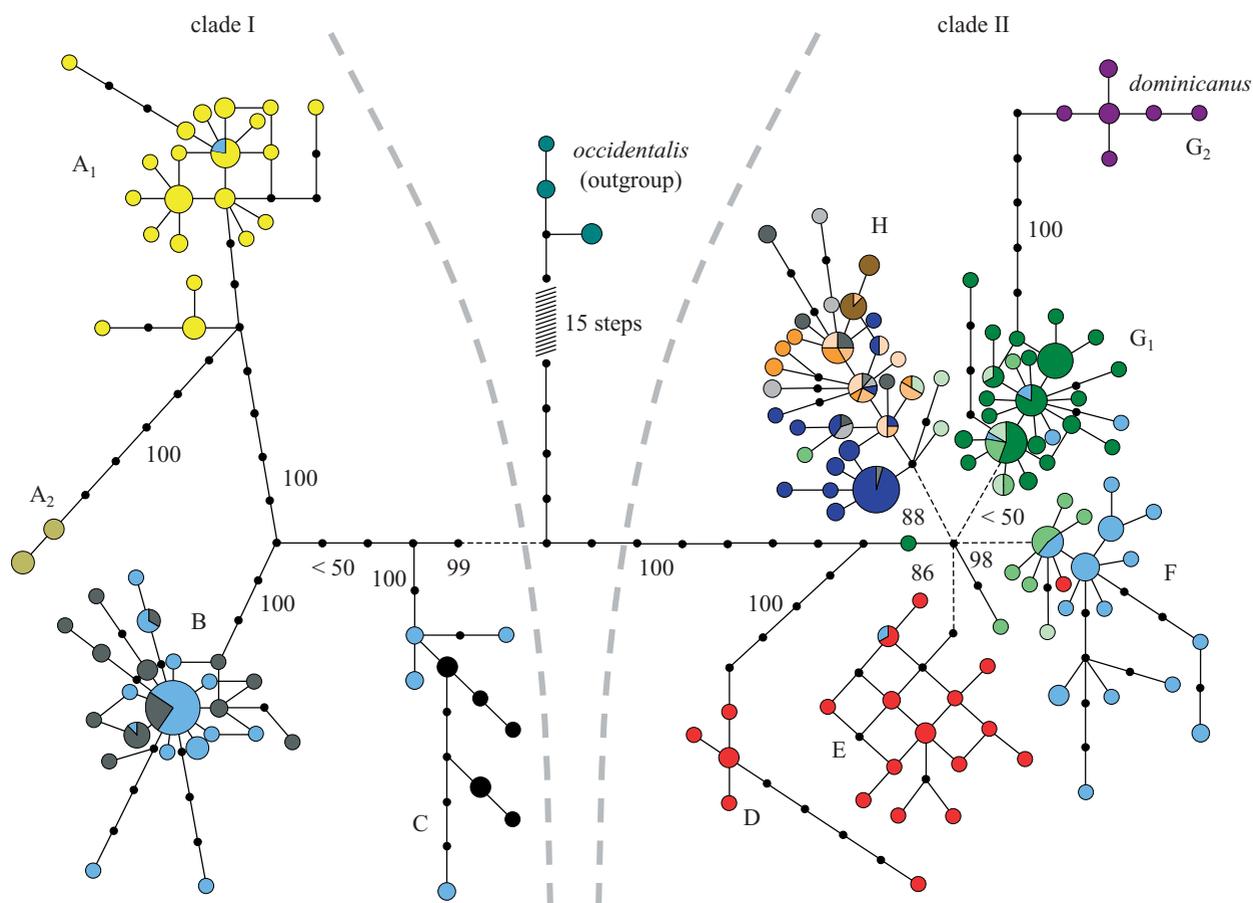
Figure 3. Median-joining network of the 160 concatenated mtDNA haplotypes (cyt *b*, control region HVR-I) identified in this study. Distinct haplotype groups are labelled A–H. *Larus occidentalis* was designated as the outgroup. Numbers along each major branch are Bayesian posterior probabilities expressed as percentages. Each (multi)coloured circle represents one haplotype, its size being proportional to the frequency of that haplotype in the entire sample. Small black dots represent inferred single-mutation steps (haplotypes not found). Stippled connections are not drawn to scale to increase clarity. Colours represent taxa as shown in figure 2.

involved. *Argentatus* haplotypes of clade I are rather basal and more widely dispersed (groups B and C) than those in clade II, probably reflecting ancient polymorphism and thus suggesting that clade I haplotypes are ancestral in *argentatus*. Nuclear amplified fragment length polymorphism markers (de Knijff *et al.* 2001) also show *argentatus* to be most closely related to clade I taxa (*atlantis* and *michahellis*). By contrast, the geographically widespread occurrence of clade II haplotypes (group F) in the extant *argentatus* population appears to be the footprint of a past gene-flow episode. Birds derived from the Aralo-Caspian refugium, possibly members of a pre-*heuglini* population, must have hybridized, perhaps briefly, with the ancestral *argentatus* population of the North Atlantic. The intro-gressed clade II mitochondrial lineage then persisted in the *argentatus* population, where it continued to diversify to this day.

The apparent polyphyly of glaucous gulls (*hyperboreus*) is more problematic because, in contrast to the situation in herring gulls, clade I and clade II haplotypes segregate geographically: in Palearctic *hyperboreus* we found only haplotypes closely related to or shared with European *argentatus* (clade I, group C), while nearctic *hyperboreus* (n = 10, all from Baffin Island) contained a variety of haplotypes shared with North American and Pacific taxa (clade II, group H). Hybridization between glaucous and

herring gulls has been observed in Iceland (Ingolfsson 1970) and northwestern Canada (Spear 1987) and may have been more frequent in the past than it is today. This may have led to strong mitochondrial introgression, per-haps approaching replacement of mtDNA of one species by that of the other (cytonuclear replacement), as seen in other cases of avian hybridization (Gill 1997; Weckstein *et al.* 2001). Nuclear markers will be needed to decide in which direction such cytonuclear replacement occurred, i.e. whether clade I or clade II haplotypes are ancestral in *hyperboreus*.

4. DISCUSSION

(a) *Evolutionary history of the herring gull complex*

What earlier authors (Geyr von Schweppenburg 1938; Mayr 1942; Haffer 1982) regarded as 'the herring gull' turned out to be an assemblage of several distinct taxa (*argentatus*, *vegae*, *smithsonianus*), which are not each other's closest relatives (figure 3). Our results show that the ring-species model does not adequately describe the evolution of the herring gull group because, contrary to the proposal of Mayr (1942), there is no overlap between the endpoints of a ring of interbreeding taxa. A circumpolar ring of interbreeding populations does exist,
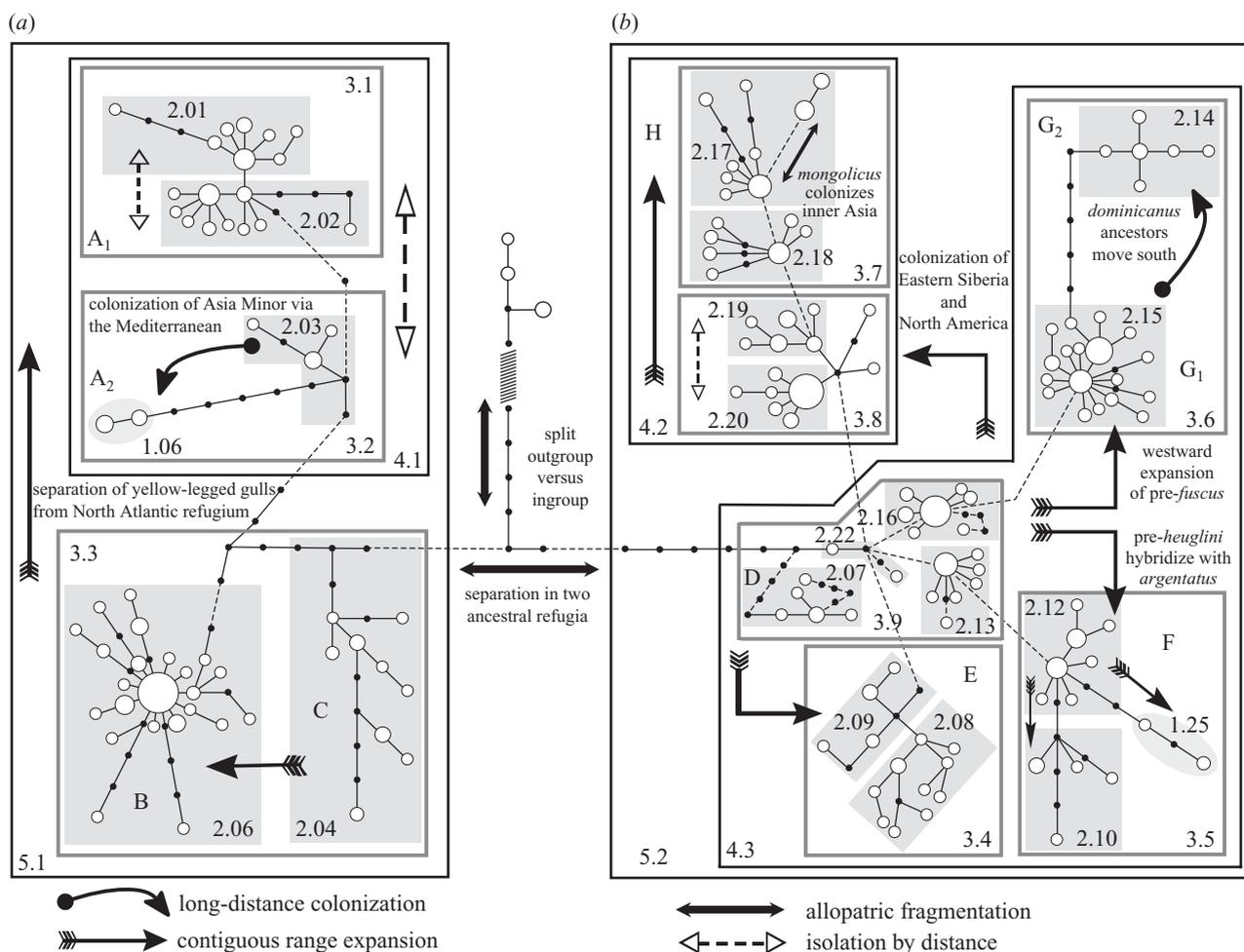
Figure 4. NCA illustrating major events in the colonization history of the herring gull complex. (*a*) Clade I and (*b*) clade II. Two-step clades are shaded; three-step clades and higher are boxed. Stippled branches of the network are not drawn to scale to increase clarity. Haplogroup designations (capital letters) correspond to those in figure 3.

Table 1. Analysis of molecular variance.
(Mitochondrial haplotypes ($n = 410$ individuals, 1571 nucleotides each) were grouped according to two different models and subjected to a hierarchical analysis of variance. Model A: groups correspond to phenotypically defined taxa; model B: in addition, geographical structure within *L. argentatus* and *L. hyperboreus* is taken into account. Haplotype frequencies for each population are given in electronic Appendix C.)

model[a]	variance component[b]	variance (%)
(A) 21 groups		
arg, arm, atl, bar, cac, dom, fus	AG: $\Phi_{CT} = 4.38$	**54.18**
glc, goi, gra, heu, hyp, int, mar	AP: $\Phi_{SC} = 1.72$	21.27
mic, mon, occ, sch, smi, tai, veg	WP: $\Phi_{ST} = 1.98$	24.54
(B) 23 groups		
arg (N of 60° N), *arg* (S of 60° N)	AG: $\Phi_{CT} = 5.70$	**71.06**
hyp (nearctic), *hyp* (Palearctic)	AP: $\Phi_{SC} = 0.34$	4.20
other taxa as in Model A	WP: $\Phi_{ST} = 1.98$	24.75

[a] Taxon abbreviations: *arg*, argentatus; *arm*, armenicus; *atl*, atlantis; *bar*, barabensis; *cac*, cachinnans; *dom*, dominicanus; *fus*, fuscus; *glc*, glaucescens; *goi*, glaucoides; *gra*, graellsii; *heu*, heuglini; *hyp*, hyperboreus; *int*, intermedius; *mar*, marinus; *mic*, michahellis; *mon*, mongolicus; *occ*, occidentalis; *sch*, schistisagus; *smi*, smithsonianus; *tai*, taimyrensis; *veg*, vegae.
[b] Variance components: AG, among groups; AP, among populations within groups; WP, within populations. Settings in ARLEQUIN: Kimura two-parameter distance with gamma correction ($\alpha = 0.03$; estimated using TREE-PUZZLE v. 5.0; Schmidt *et al.* 2000); 10 000 random permutations of sequences among populations. All Φ values are significant at $p < 0.001$.

but it is made up entirely of taxa belonging to mitochondrial haplotype clade II, and its endpoints do not overlap. Current coexistence in sympatry, e.g. between *argentatus*

and *fuscus* in Europe or between *marinus* and *smithsonianus* in North America, occurs between clade I and clade II taxa and is caused not by 'circular overlap', but by second-
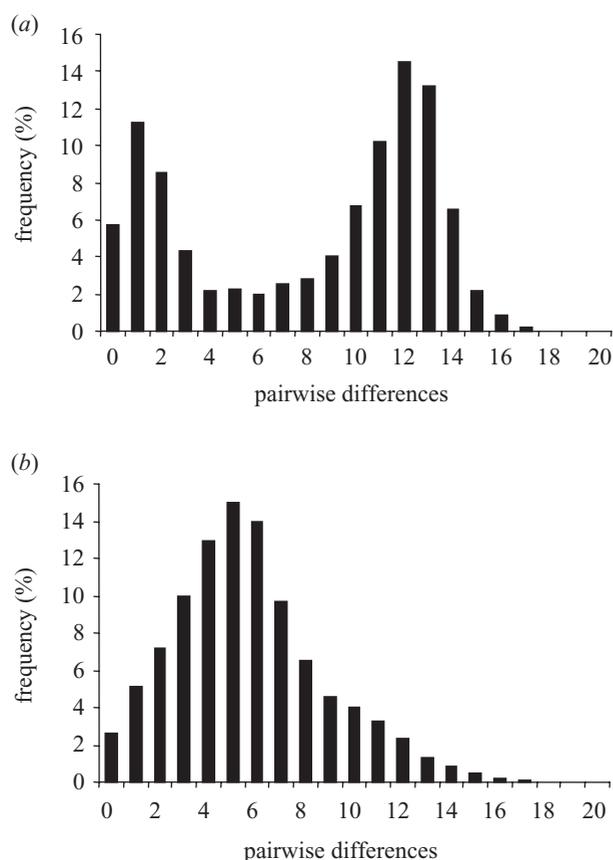
Figure 5. Frequencies of pairwise sequence differences between haplotypes ('mismatch distributions') within (*a*) clade I and (*b*) clade II of the mitochondrial haplotype network.

ary sympatry between forms that diverged in allopatry. Thus, not isolation by distance, but vicariance and subsequent range expansion, and also in some instances (*armenicus*, *dominicanus*) long-distance colonization events, were the processes that played the decisive role in the evolution of the herring gull complex.

The current mitochondrial genetic make-up of the herring gull (*argentatus*) shows clear signs of past hybridization between birds derived from different (clades I and II) ancestral refugia, although no definite geographical scenario can be reconstructed for this reticulation. Such polyphyletic representation in the haplotype network (also evident in *hyperboreus*) provides a striking illustration of how discrepancies may arise between a gene tree and the taxon phylogeny. The fact that some species, apparently owing to past gene-flow episodes, contain highly divergent haplotypes suggests that mitochondrial lineage sorting may have quite different and unpredictable outcomes: for instance, in the distant future only haplotypes of one major clade (I or II) may survive in *argentatus*, but we cannot predict which. Once lineage sorting is complete, *argentatus* will appear in the mitochondrial gene tree to be most closely related either to *marinus* and *michahellis–atlantis* (clade I) or to *cachinnans* and *fuscus* (clade II). Nuclear markers (de Knijff *et al.* 2001) indicate that the first scenario is historically correct.

Although *Larus* gulls do not currently fulfil the essential criteria of a ring species, this situation may be about to change: lesser black-backed gulls (*L. fuscus graellsii*) are

expanding westwards and currently breed as far west as Greenland (Boertmann 1994). The species may soon colonize North America, where many birds are already observed annually (Post & Lewis 1995). It will be interesting to see whether *graellsii* will turn out to be reproductively isolated from North American *smithsonianus*. If so, the circumpolar ring of interbreeding taxa might yet close to form a classic ring species, not by an invasion of herring gulls from North America to Europe as postulated earlier (Geyr von Schweppenburg 1938; Mayr 1942), but by expansion of lesser black-backed gulls in the opposite direction.

(b) Genetic divergence versus reproductive isolation

Although our results do not support a speciation model involving only isolation by distance, the origin of reproductive barriers in the herring gull complex does provide important insights into the speciation process. An ancestral population may split up into two or more daughter populations, which gradually acquire reproductive isolation over a prolonged period, but at different rates (Grant & Grant 1997). In fact, we found no close relationship between mitochondrial genetic distance, which is roughly proportional to time since divergence, and reproductive isolation. The most divergent taxon in terms of mtDNA, *L. occidentalis*, is known to hybridize extensively with one of the ingroup taxa, *L. glaucescens*, along the west coast of North America (Bell 1997). On the other hand, our data do not support the traditional view of the greater black-backed gull (*L. marinus*) being an outgroup relative of the herring gull complex. Although *L. marinus* is fully reproductively isolated from all species it co-occurs with, in the mtDNA network it is nested among several taxa that hybridize: *argentatus* × *hyperboreus* (Ingolfsson 1970; Spear 1987), *michahellis* × *graellsii* (Van Swelm 1998), *cachinnans* × *argentatus* (Panov & Monzikov 1999) and earlier in the twentieth century *argentatus* × *fuscus* (Tinbergen 1953). Assuming equal mutation rates among lineages, reproductive isolation of *L. marinus* from its currently sympatric congeners evolved more rapidly than, for instance, between *L. occidentalis* and *L. glaucescens*. This must have involved a period of geographical isolation, probably in northeastern North America (cf. figure 1*b*).

We hypothesize that *marinus* diverged in allopatry from the rest of clade I, although NCA indicates not allopatric fragmentation but contiguous range expansion for this split. Two factors could explain this discrepancy.

(i) We sampled *marinus* only in its Palearctic, not its Nearctic, range; broader sampling may alter the NCA inference.

(ii) If *marinus*, a taxon characterized by haplotypes that are relatively basal in the network, secondarily expanded its range to the Palearctic, where it now overlaps extensively with *argentatus*, NCA (based on current ranges) may no longer recover the historical pattern. Similar limitations of NCA have been identified in a study of an Iberian lizard (Paulo *et al.* 2002).

A survey involving experimental cross-mating between *Drosophila* species showed that, for a given genetic

distance, the degree of reproductive isolation is much greater between sympatric than between allopatric species, indicating that reinforcement plays a role in the evolution of reproductive isolation (Coyne & Orr 1997). If this is also true in gulls, it could mean that *L. marinus*, after a period of allopatric divergence, has had a relatively long history of geographical contact with closely related taxa (*hyperboreus*, *argentatus*, *atlantis*, and later with *smithsonianus* and *fuscus*), which facilitated the perfection of reproductive barriers through reinforcement. This may explain why *L. marinus* achieved complete reproductive isolation more rapidly than other taxa in the herring gull group and, thus, why the phylogenetic age of the *marinus* lineage was previously overestimated. Novel theoretical considerations (Servedio & Saetre 2003) also ascribe a more important role to reinforcement than was hitherto accepted (Turelli *et al.* 2001).

We conclude that the establishment of reproductive barriers, several of which are still incomplete, occurred at different rates and allowed for some reticulation between lineages, which is reflected in the mitochondrial phylogeny. The degree of reproductive incompatibility, therefore, can serve only as a rough indicator of the phylogenetic distance between gull taxa. This agrees with findings in *Drosophila* (Coyne & Orr 1997), *Plethodon* salamanders (Highton 1995) and pigeons (Lijtmaer *et al.* 2003), which show that, although there is a general correlation between genetic divergence and the degree of postzygotic isolation, this correlation is far from perfect, i.e. in some cases strong isolation occurs even between species that are poorly diverged genetically.

(c) Significance of the ring-species model

Good examples fulfilling the stringent criteria of the ring-species model appear to be rare (Irwin *et al.* 2001*a*). The conclusions we derived here for the herring gull complex are similar to those reached in studies of other potential ring species, for example the *Ensatina* complex of salamanders in western North America (Wake 1997) and the great tit *Parus major* complex in Eurasia (Kvist *et al.* 2003). In both cases, ancestral populations expanded in a roughly circular fashion around an uninhabitable area, but there have been intermittent periods of allopatric fragmentation and subsequent range expansion. This led to areas of secondary contact, where hybridization currently occurs, often in very narrow zones. Population divergence, therefore, proceeded at least partly in allopatry, not exclusively through isolation by distance throughout a contiguous range.

A case that closely approximates a true ring species is that of the Asian greenish warbler *Phylloscopus trochiloides* group (Irwin *et al.* 2001*b*). Ancestors of this complex spread from south of the Himalayas east and west around the Tibetan Plateau, north of which the ring closed between two taxa that are reproductively isolated, primarily through divergent songs that function in mate recognition. Song evolution is driven by sexual selection, which may produce rapid divergence. So reproductive divergence in these warblers seems to have come about through a combination of isolation by distance and sexual selection. The main difference between this and the herring gull case is that the ring-shaped range of the warblers has closed, leading to geographical overlap between the two terminal taxa, whereas in the gulls this is not (yet) the case.

In conclusion, although ring speciation is theoretically possible, the few well-studied examples suggest that it occurs infrequently, because the dynamics of species' ranges are more likely to result in fragmentation, i.e. periods of allopatry, before the slow process of isolation by distance leads to sufficient divergence to allow for circular overlap.

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