Molecular phylogeny and plumage evolution in gulls (Larini)

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Abstract

We used DNA sequence data of the mitochondrial control region and cytochrome *b* gene to assess phylogenetic relationships among 32 gull species and two outgroup representatives. We tentatively estimated divergence times from transversional substitutions calibrated against DNA–DNA hybridization data. Several strongly supported species groups are identified, but the relationships between these species groups and the rooting of the gull tree remain unresolved. Geographical range extension appears as a factor of speciation, but several related, well-differentiated species seem to have evolved within comparatively restricted areas. Some plumage characters used in the past for delimiting species groups appear inappropriate. The dark hooded species, for instance, do not constitute a natural assemblage. Molecular data also allowed the identification of several striking plumage convergences that had obscured the true relationships between gull species until now. For example, the dark tropical gulls analysed here each belong to totally different clades and are independent examples of convergent plumage evolution under common environmental constraints. The reverse situation also happened, with two arctic-distributed species, the ivory gull (*Pagophila eburnea*) and the Sabine's gull (*Xema sabini*), appearing as sister taxa despite completely different plumage features. Molecular data have thus significantly improved our understanding of gull evolution.

Introduction

The gulls (tribe Larini) constitute a well-delimited, cosmopolitan group of birds including 50 extant species (following Sibley & Monroe's, 1990, systematic treatment). They all share a similar general outlook, being medium-sized with nonspecialized bills, webbed feet, short legs and neck. They are all colonial, long-lived species, and some are amongst the most intensively studied birds (see for instance applied aspects in Blokpoel & Scharf, 1991; Isenmann *et al*., 1991; Meathrel *et al*., 1991; Spaans *et al*., 1991; Vermeer & Irons, 1991).

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Gulls are widespread from tropical to arctic regions and, although all species are closely associated with wetlands or marine environment both during and out of the reproductive season, they have evolved under highly different ecological constraints and have adapted to many different habitats (del Hoyo *et al*., 1996). This widely studied group thus shows enough diversity in several life-history or morphological traits for comparative analyses of these traits to be especially interesting (Jouventin & Mougin, 1981).

A reliable phylogeny is a prerequisite for such analyses. In the past, three main studies have addressed the question of the relationships within the Larini, based, respectively, on plumage (Dwight, 1925), behaviour (Moynihan, 1959) and morphometrics (Schnell, 1970a, b). Recently, Chu (1998) published a complete cladistic analysis based on 117 skeleton and 64 integument characters. Further hypotheses concerning the relationships

within particular subsets of species have been put forward (see Cramp & Simmons, 1983, or del Hoyo *et al*., 1996). Despite this considerable interest, no general consensus exists on a phylogenetic arrangement of Larini. Most gulls are generally included in the genus *Larus* except several species believed to have diverged first (see del Hoyo *et al*., 1996). Within the genus *Larus*, two main groups have been long recognized (e.g. Dwight, 1925; Moynihan, 1959; Cramp & Simmons, 1983; Sibley & Monroe, 1990; del Hoyo *et al*., 1996). One includes large, white-headed species, the other small species having a dark hood or believed to have recently lost their hood. Several species have been variously assigned to either of these groups in previous treatments of gull relationships. This split between white-headed and black-headed species has recently been questioned by Chu (1998), who suggested instead a basal split between 'larine' gulls and 'sternine' gulls (this last group made of the species usually classified in genera other than *Larus* as well as some black-headed species). In Table 2 and Fig. 3, we present the taxonomic relationships between the species that we analysed according to Dwight (1925), Moynihan (1959) and Chu (1998). The phenetic analysis of Schnell (1970a,b) was excluded because this author clearly stated in his papers that his aim was not to reconstruct phylogenetic relationships among gulls.

The lack of consensus that is apparent when comparing the results of these studies and the uncertainty surrounding the relationships of several species leaves room for a molecular investigation of the phylogeny of the Larini based on DNA sequence data. Examples of such investigations, generally based on mitochondrial genes, are now numerous in birds (see Mindel, 1997, for reviews and examples). In the present study, we sequenced parts of the mitochondrial control region and of the cytochrome *b* gene. The cytochrome *b* gene is one of the most widely used for phylogenetic studies at the genus/family level. The mitochondrial control region has mainly been employed at the species level, but also once at the genus level (Marshall & Baker, 1997). It is generally a fast evolving gene, with its ETAS domain and CSB domain being hypervariable regions whereas the central domain is less variable (Baker & Marshall, 1997; Sbisà *et al*., 1997). In gulls, preliminary investigations showed that its level of differentiation, even between widely divergent species, was adequate for phylogenetic purposes. For the present work, we sequenced the central and CSB control region domains and a short segment from the cytochrome *b* gene.

According to DNA–DNA hybridization-based avian systematics (Sibley & Ahlquist, 1990), the closest relatives of gulls within the Charadrii are the terns (*Sterna* and allied genera). We therefore sequenced specimens of two tern species and used published sequences from another Charadriiformes as outgroup representatives.

We analysed samples from 32 gull species, covering most species groups, to obtain a rather complete picture of species relationships within the Larini. Only the Ross's gull (*R. rosea*) and Dolphin gull (*L. scoresbii*) were missing among the species with uncertain affinities, while no member of the *L. crassirostris* – *L. belcheri* – *L. pacificus* assemblage could be included.

Materials and methods

Tissue samples and DNA extractions

The origin of the sequenced specimens is detailed in Table 1. Samples were plucked feathers, muscles or blood in buffer or ethanol, skin or feathers from long-dead bodies, dried plucked feathers, dried blood on paper, or for museum specimens skin from the underside of the foot. DNA was extracted from most fresh samples by complete digestion in 5% Chelex 100 (Biorad, Hercules, USA) with 20 μL of proteinase K followed by a 10-min boiling. Extractions from blood samples were performed using Qiaamp tissue extraction kit (Qiagen, Santa Clarita, CA, USA) following the supplier's procedure. Most museum specimens were processed with a silica method adapted from Taberlet & Fumagalli (1996), adding proteinase K to the extraction buffer in the sample digestion step.

Amplification and sequencing

Polymerase chain reaction (PCR) amplifications were carried out in 50-μL volumes containing 1× amplification buffer/1 unit of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.4 μM of each primer. Direct sequencing was performed on an automated sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) following recommended procedures. We checked the accuracy of our sequencing procedure by independent amplification and sequencing of the whole control region segment in 10 individuals of five unrelated species (see Results). We subsequently sequenced one strand for each specimen, generally producing largely overlapping segments. The amplification primers for parts II and III of the control region were L438 (5′-TCACGTGAAA TCAGCAACCC-3′) and H1248 (5′-CATCTTCAGTGCCA TGCTTT-3′). For museum specimens, three overlapping segments of the control region were amplified separately, using primers L438 and HDLI3 (5′-GTATTCCTGAGGGC CAAACT-3′), L699 (5′-ATAAACCCCTCCAGTGCACC-3′) and HHTR (5′-ATCGCTGTTGTTGACATGTA-3′) and L892 (5′-GTGTAGTGCTCAATGGACATG-3′) and H1248. The sequencing primers were L438, L699 and L892. Part of the cytochrome *b* was amplified using primers L15008 (5′-AACTTCGGATCTCTACTAGG-3′) and H15326 (5′-GAATAAGTTGGTGATGACTG-3′), which were also used as sequencing primers. L refers to light and H refers to

Table 1 Number of specimens used per species and geographical origin of specimens from which DNA samples were analysed (MNHN = Muséum National d'Histoire Naturelle, Paris; LSUMZ = Louisiana State University Museum of Natural Science).

Black-legged kittiwake *(Rissa tridactyla)*, 1, Brittany, France, Etienne Danchin.
Red-legged kittiwake (*Rissa brevirostris*), 1, Buldir Island, Alaska, Jeff Williams, courtesy Richard Bradbury.
Ivory gull (*Pagophila eburnea*), 1, Groenland, MNHN 1911-978.
Sabine's gull (*Xema sabini*), 1, Royans, France, MNHN 1930-460.
Swallow-tailed gull (*Creagrus furcatus*), 1, Alaza Island, Galapagos, MNHN 1970-864.
Little gull (*Larus minutus*), 1, Biarritz, France, MNHN 1990-747.
Franklin's gull (*Larus pipixcan*), 1, Punta Canero, Guayas, Ecuador, Zoological Institute of the University of Copenhagen.
Laughing gull (*Larus atricilla atricilla*), 1, Grand Connétable Island, French Guyana, Olivier Tostain.
Mediterranean gull (*Larus melanocephalus*), 2, Camargue, France, Pierre Defos du Rau/Tour du Valat.
Andean gull (*Larus serranus*),1, Chimborazo, Ecuador, Zoological Institute of the University of Copenhagen.
Bonaparte's gull (*Larus philadelphia*), 1, offshore, Grays Harbor, WA, USA, LSUMZ B 21799.
Slender-billed gull (*Larus genei*), 2, Camargue, France.
Black-headed gull (*Larus ridibundus*) 12, Dombes and Forez areas, France, Anne-Caroline Prévot-Julliard.
Red-billed gull (*Larus scopulinus*), 1, South Island, New Zealand, Zoological Institute of the University of Copenhagen.
Grey-headed gull (*Larus cirrocephalus cirrocephalus*), 2, Guayas, Ecuador, Zoological Institute of the University of Copenhagen.
Great black-headed gull (*Larus ichthyaetus*), 1, Kuwait, LSUMZ B-15467.
Yellow-legged gull (*Larus cachinnans michahellis*), 10, Camargue, France.
Herring gull (*Larus argentatus argenteus*), 7, Brittany, France, Pierre Yésou.
Lesser black-backed gull (*Larus fuscus graellsii*), 2, Brittany, France, Pierre Yésou.
Great black-backed gull (*Larus marinus*), 2, Brittany, France, Pierre Yésou.
Iceland gull (*Larus glaucoides glaucoides*), 1, Nolsoy, Færoe Islands, Zoological Institute of the University of Copenhagen; (*Larus glaucoides kumlieni*), 1, Coats Island, North West Territory, Canada, Tony Gaston, courtesy Richard Bradbury; (*Larus glaucoides thayeri*), 1, Cameron Parish, LA, USA, LSUMZ b-21816.
Glaucous gull (*Larus hyperboreus hyperboreus*), 2, Russia, Swedish Museum of Natural History NRM 946577 and NRM 946581.
Yellow-footed gull (*Larus livens*), 2, San Pedro Martir Island, Mexico, Bernie Tershy, courtesy Richard Bradbury.
Western gull (*Larus occidentalis occidentalis*), 1, Grays Harbor, WA, USA, LSUMZ B-20480.
Kelp gull (*Larus dominicanus*), 2, Kerguelen Islands, MNHN 1951-668 and South Island, New Zealand, Martin Renner.
California gull (*Larus californicus*), 2, Mono Lake, CA, USA, Joseph Jehl, courtesy Richard Bradbury.
Ring-billed gull (*Larus delawarensis*), 2, Lake Ontario, Canada, Chip Weseloh.
Common gull (*Larus canus canus*), 1, Nolsoy, Færoe Islands, Zoological Institute of the University of Copenhagen.
Audouin's gull (*Larus audouinii*), 5, Ebro Delta, Spain, A. Johnson, courtesy Y. Kayser and M. Genovart.
Sooty gull (*Larus hemprichii*), 1, Hormuz Strait, MNHN (no specimen number).
Heermann's gull (*Larus heermanni*), 1, Grays Harbor, WA, USA, LSUMZ B-20534.
Grey gull (*Larus modestus*), 1, Guayas, Ecuador, Zoological Institute of the University of Copenhagen.
Cayenne tern (*Sterna sandvicensis eurygnatha*), 1, Grand Connétable Island, French Guyana, Olivier Tostain.
Royal tern (*Sterna maxima*), 1, Grand Connétable Island, French Guyana, Olivier Tostain.

heavy strands, and the numbers refer to the position of the 3′ end nucleotide of the primer in the white Leghorn chicken (*Gallus gallus*) mtDNA sequence (Desjardins & Morais, 1990).

Phylogenetic analyses

A composite segment including the control region and cytochrome *b* segments was aligned manually

and subjected to phylogenetic analysis. The control region and cytochrome *b* sequences were first treated separately, then combined in a composite segment. The level of saturation in the data set was checked by plotting the number of transitions vs. the number of transversions for all pairs of haplotypes. Phylogenetic trees were reconstructed using the maximum-likelihood (ML) method, the maximum-parsimony (MP) method (DNAML and DNAPARS in the PHYLIP package, version 3.57c; Felsenstein, 1993) and the neighbour-joining (NJ) method using distance matrix based on the Kimura 2-parameters model (MEGA; Kumar *et al*., 1993). We computed the likelihood of the ML tree for several integer values of the transitions/transversions ratio ranging from 2 to 10. The likelihood was maximal when this ratio was equal to 4, and we used this value to re-estimate the ML tree (Felsenstein, 1993). All three methods gave near-identical topologies (see Results). We evaluated the robustness of the trees by bootstrap (Felsenstein, 1985) (500 replications), based for the sake of computational rapidity on the NJ method. We also performed the NJ method using only transversions to try to elucidate the basal relationships within Larini. All different haplotypes of a same species always clustered together except for *L. hyperboreus* and *L. glaucoides*. For *L. hyperboreus*, the partial sequencing of 10 further individuals showed that one of the two original specimens had an atypical haplotype, possibly as a result of hybridization with *L. argentatus*. We retained the most frequent (11 out of 12) *L. hyperboreus* haplotype for phylogenetic analysis. *L. glaucoides glaucoides* and *L. glaucoides kumlieni* did branch together while *L. glaucoides thayeri* did not. We thus retained *L. g. thayeri* and *L. g. glaucoides* for the various treatments. We randomly selected one haplotype per species for all other species. Because of missing data, the position of *L. hemprichii* was determined by the NJ method using a reduced data set. We used sequences of Dunlin (*Calidris alpina*) retrieved from GeneBank (accession number U34686 for the cytochrome *b* and L20137 for the control region) to obtain another outgroup.

Molecular clock calculations

In birds, DNA sequence divergence is often calibrated against the DNA–DNA hybridization data of Sibley & Ahlquist (1990). Moum *et al*. (1994) used for calibration 1 unit of $\Delta T_{50}H$ per 3.0 million years for bird species with an age at first breeding larger than 2 years. This rate dates the divergence between *Larus* and *Sterna* ($\Delta T_{50}H = 4.5$) back to 13.5 million years ago (MYA). The evolution rate of our mtDNA sequences was then estimated using distances based on transversions only for the complete data set (Kimura two-parameter model in the MEGA package; Kumar *et al*., 1993). Although dating of cladogenesis based on calibration of a molecular clock is questionable (e.g. Li & Graur, 1991), we provide tentative estimates of divergence times to be compared with previous studies.

Results

Sequence length and evolution

The check of the sequencing protocol based on 10 individuals of five species (see Methods) showed only one base position with an unambiguous difference out of more than 6000 positions examined. Due to heteroplasmy in the number of tandem repeats at the end of the control region (CAACAAA in the L strand in most gull species analysed, as in many other Ciconiiformes; Berg *et al*., 1995), we could generally not sequence the whole amplified control region fragment. We obtained a 640–650 bp long segment for all species but *L. hemprichii*, for which the segment between L699 and HHTR could not be amplified because of an insertion in the annealing site of the L699 primer. The sequenced cytochrome *b* segment was 275–300 bp long. The concatenation of the two regions yielded a data set of 935 nucleotide positions, 275 for the cytochrome *b* and 660 (including indels) for the control region, available for 31 gulls and the two *Sterna*, except a few ambiguous sites in some species. Only 650 bp were sequenced for *L. hemprichii*. They were 317 variable sites and 246 informative sites in the overall data set, 243 and 149, respectively, when terns were excluded. The control region evolved on average slightly faster than cytochrome *b* (mean value for the ratio of pairwise distances 1.27) when terns were included in the data set but, within gulls, the mean evolution rates of control region and cytochrome *b* segments were equal. Several gull clades even exhibited a slow rate of evolution of the control region compared with cytochrome *b*. While this is not unusual for the central part of the control region, it is surprising that the generally hypervariable control region part III did not evolve faster than the cytochrome *b* segment (Baker & Marshall, 1997). Details and possible explanations for these findings will be discussed in a separate paper. The graph of the number of transitions plotted against the number of transversions for the control region only (Fig. 1) indicated a significant amount of saturation between pairs of species belonging to different groups (as determined by this analysis) and between the Larini and the outgroup species. No saturation was apparent for the cytochrome *b* segment (not shown). Most substitutions in the cytochrome *b* concerned third positions (69 vs. 12 in first and one in second positions) and were synonymous mutations (71 out of 82). Percentage divergence (Kimura two-parameter model) between gull species based on all substitutions ranged from 0.1% (one position) between *L. cachinnans* and *L. marinus* to 10.8% between *L. ichthyaetus* and *X. sabini*. The divergence between the two tern species and the

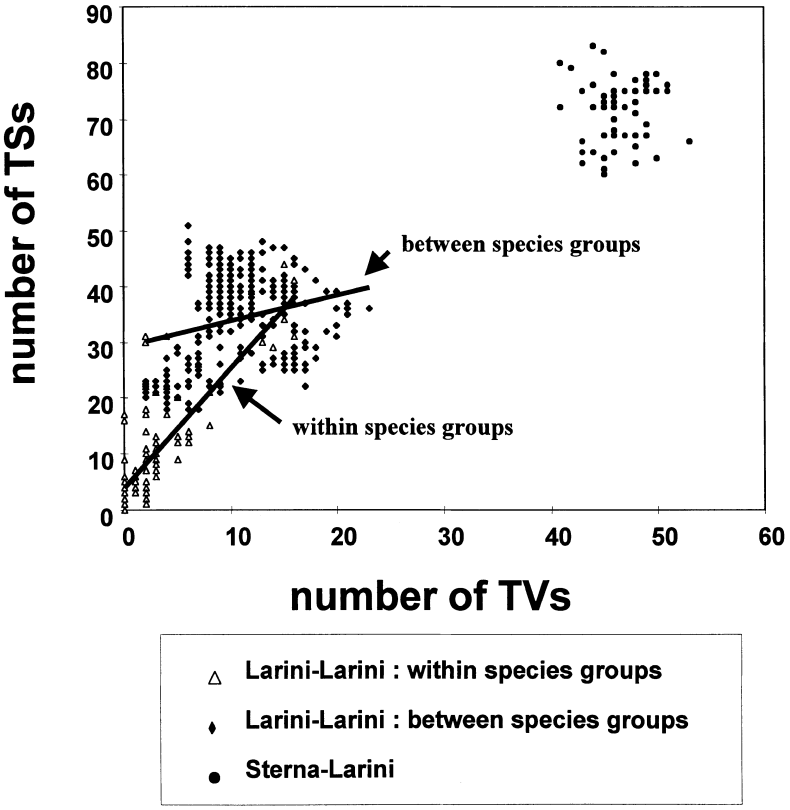

Fig. 1 Number of transitions plotted against the number of transversions within a 660-bp-long segment of the mitochondrial control region of Larini. Each point represents one pairwise comparison. *L. hemprichii* was excluded from the present analysis. Linear regression lines for comparisons within and among species groups are indicated.

gulls varied between 17.6% and 21.4% for all substitutions.

Phylogeny

Analysis of our data set yielded several well-defined clades of which the basal relationships remained largely unresolved (Fig. 2). These clades can be organized into eight main species groups of comparatively recent origin identified by all methods (ML, MP and NJ) when applied to the combined data set and supported by high bootstrap scores (90–100).

These eight species groups were (names follow past usage in ornithological literature):

1 the 'white-headed' species (*L. argentatus*, *L. marinus*, *L. fuscus*, *L. cachinnans*, *L. dominicanus*, *L. californicus*, *L. hyperboreus*, *L. glaucoides*, *L. occidentalis*, *L. livens*, *L. delawarensis*, *L. canus*, with *L. heermanni);*

2 the 'hooded' species (*L. pipixcan* and *L. atricilla*, with *L. modestus*);

3 the 'black-headed' species (*L. melanocephalus* and *L. ichthyaetus*, with L. audouinii and *L. hemprichii*);

4 the kittiwakes (*R. tridactyla* and *R. brevirostris*);

5 the arctic species (*P. eburnea* and *X. sabini*);

6 the little gull (*L. minutus*);

7 the swallow-tailed gull (*C. furcatus*);

8 the 'masked' species (*L. genei*, *L. philadelphia*, *L. scopulinus*, *L. serranus*, *L. cirrocephalus*, *L. ridibundus*).

The little gull and swallow-tailed gull clustered together in all three methods but with a low bootstrap score and might better be regarded provisionally as two independent lineages.

An analysis restricted to the control region yielded the exact same groups but with lower bootstrap values, indicating that the cytochrome *b* sequences provided congruent information compared with the control region. Indeed, the noncongruent nodes in the tree based on the cytochrome *b* sequences only were supported by very low bootstrap scores (4–17), indicating that discrepancies between the two partial data sets could reasonably be attributed to the shortness of the cytochrome *b* segment sequenced.

The branching order between the eight species group was nearly identical under the ML, NJ and MP methods. The only difference is that the little gull and the swallow-tailed gull were the closest relatives of groups 1, 2 and 3 under the MP and ML methods but not under the NJ method. The position of the terns relative to the gull species groups also differed: under the NJ method, the kittiwakes occupied a basal position relative to other gulls, whereas ML and MP methods identified the 'masked' species group (*L. ridibundus* and its relatives)

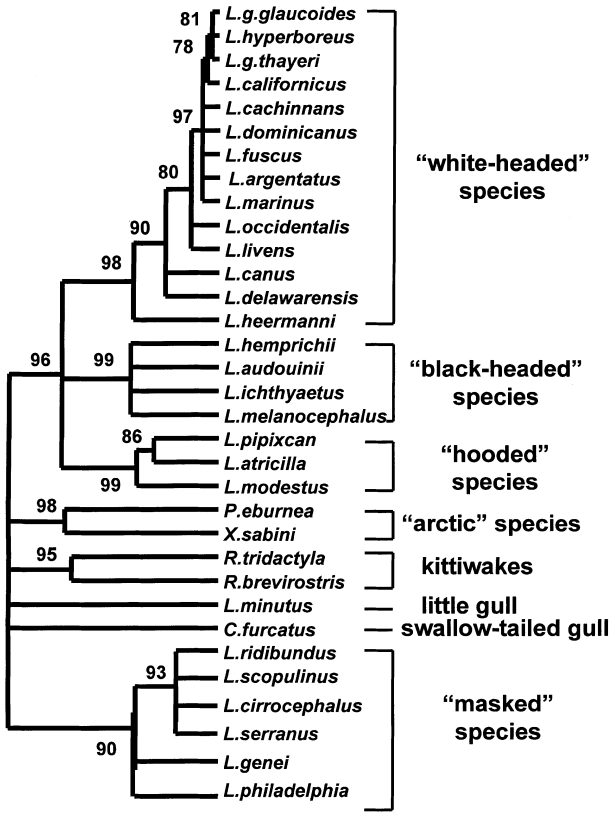

Fig. 2 Our phylogenetic hypothesis for the 32 gull species analysed. This tree is derived from the neighbour-joining tree by collapsing all internal branches supported by bootstrap scores under 75. In addition, we collapsed branches unifying *L. marinus* and *L. cachinnans* because we feel our data are not informative enough at such small-scale genetic differentiation. Branch lengths were manually adjusted to be broadly proportionate to relative divergence time. We have indicated to the right the name of the species groups used in the text. Bootstrap scores for the retained nodes have been reproduced.

as the first split within gulls. In any case, the position of the outgroup and the relationships between gull species groups were supported by low bootstrap scores (17–45). Only the node linking the 'hooded' species group (*L. atricilla* – *L. pipixcan* – *L. modestus*) and the 'black-headed' species group (*L. melanocephalus* – *L. hemprichii* – *L. audouinii* – *L. ichthyaetus*) with the 'white-headed' species group 1 was strongly supported (bootstrap score always over 95 with the NJ method). Whereas the saturation of the control region sites was unlikely to have affected the identification of these eight species groups (as shown by comparisons, Fig. 1, between species within species groups), it may have been responsible for the uncertain relationships between them and between gulls and terns. We performed the same analyses on transversions only but failed to increase the robustness of the basal nodes of the trees.

The resolution of the branching pattern within these clades was uneven. Among the 'white-headed' species group, several well-supported nodes pictured a clear evolutionary history. *L. heermanni* was identified as the most divergent species, followed by *L. canus* and *L. delawarensis* originating from more recent splits. The *L. argentatus* complex in the broad sense constituted a very homogeneous assemblage (percentage divergence 0.1–1.4) with *L. occidentalis* – *L. livens* and *L. hyperboreus* – *L. glaucoides* appearing as distinct lineages. Within the 'hooded' species group, *L. pipixcan* and *L. atricilla* appeared as well supported sister species relative to *L. modestus*. *L. genei* and *L. philadelphia* constantly occupied a basal position within their 'masked' species group, while not being sister species in the ML or MP methods. The topology of the NJ tree concerning relationships within the 'black-headed' species group was not retained under ML or MP methods.

The monophyly of the gulls was checked by using the Dunlin as outgroup. The terns were then excluded from the gulls clade by all three methods, with bootstrap value of 100 under the NJ method. It seems unlikely that the remaining gull species that we did not analyse would change this result, since none of these appears as especially differentiated compared with the species we used.

Considering the lack of resolution of ancient nodes and, in contrast, the strong support for species groups, we will base the discussion on a consensus tree, retaining only nodes supported by a bootstrap score of over 75 (Fig. 2).

Estimation of divergence times

The mean estimated divergence based on transversions only between *Sterna* and the Larini was 6.30%, leading to a calibration of 0.47% divergence per million years. The mean divergence estimates between the six clades originating from the basal split of the consensus tree (Fig. 2) ranged from 1.20 to 2.79%. Neglecting the ancestral polymorphism (=within-species variability), which appeared to be quite small on the basis of our results, this dated the first split within Larini back to 2.6–5.9 MYA. Most extant species would have originated within their species group during the last million years, except for the kittiwakes (*R. tridactyla* – *R. brevirostris*) and the arctic species (*P. eburnea* – *X. sabini*) where the species would have last shared a common ancestor around 2.0 MYA.

Discussion

Comparison with previous phylogenetic hypotheses

Our results confirmed the finding of Chu (1998) concerning the profound division of the hooded species group into two main lineages that cluster at the base of the gulls clade. Although uncertainties still exist in the relative order of divergence of the species groups

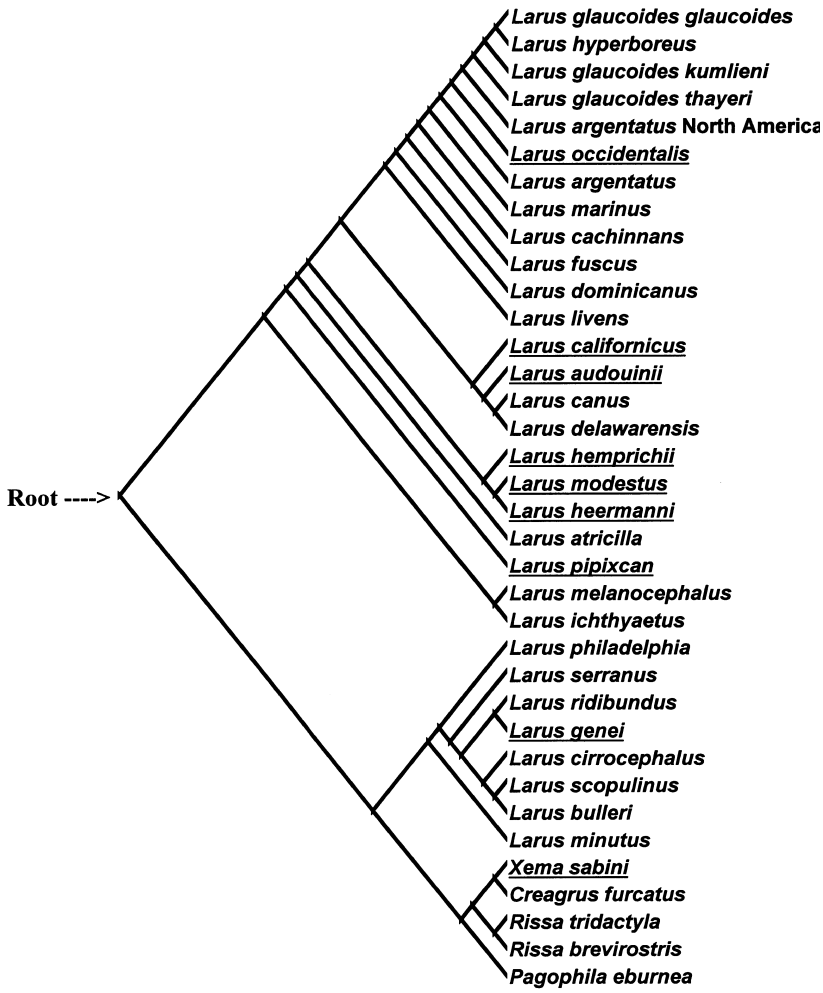

Fig. 3 The single shortest tree found by Chu (1998) after parsimony analyses of 117 osteological and 64 integumentary characters. Only the species analysed in our work are depicted. Species underlined seem to be incorrectly positioned according to our results.

revealed by this work, the *L. genei* – *L. ridibundus* species group, *L. melanocephalus* and *L. ichthyaetus*, *L. atricilla* and *L. pipixcan*, and *L. minutus*, belong to totally different clades. When turning to the composition of the species group, though, Chu's analysis did not perform significantly better than that of Moynihan (1959), which combined behavioural and phenetic data, although both analyses provide a better picture of gull evolution than the purely intuitive approach of Dwight (1925).

Both Chu and Moynihan failed to uncover the true relationships between the members of Moynihan's 'primitive hooded' species (see Table 2). They were also unable to identify several cases of convergence of plumage characters. One of the most striking of these concerns the Audouin's gull, *L. audouinii*. This species has always been considered as related to the large white-headed group, and a superspecies relationship with *L. canus* and *L. delawarensis* has even been suggested (see Sibley & Monroe, 1990). In fact, our results establish that *L. audouinii* is an independent and isolated realization of the 'large white-headed species' morphological model, possibly related to its fish diet. A white head has been considered as a possible adaptation for increased fishing efficiency (Isenmann, 1976). Unexpected also was the sister taxa relationship of *P. eburnea* and *X. sabini*, which is nevertheless one of the best-supported nodes in our results. *X. sabini* had previously been tentatively related to *C. furcatus* by Moynihan and by Chu, on the basis of similar plumage and bill coloration. Harris (1970) nevertheless refuted this opinion. *P. eburnea* seems to have always been treated as an original form without close relative. Other examples of misleading plumage characters are the dark tropical species *L. hemprichii*, *L. modestus* and *L. heermanni* (see below).

In fact, other characters point toward the true relationships between these convergent species, but they have been dismissed because of the striking plumage similarities. For example, the powerful bill and heavy build of *L. heermanni* best match those of the large species which are its closest relatives, while *L. modestus* is closer in this respect to *L. pipixcan* and *L. atricilla* (Dwight, 1925). Furthermore, Moynihan

Table 2 Taxonomic relationships among the species analysed in this work according to Dwight (1925) and Moynihan (1959). Species names are those of Sibley & Monroe (1990) for the sake of simplicity, not necessarily those used in the summarized papers.

Dwight (1925)	Moynihan (1959)
Genus *Larus*	Subgenus *Larus*
Subgenus *Larus*	The white-headed species
L. glaucoides	Subgroup
L. hyperboreus	*L. glaucoides*
L. marinus	*L. hyperboreus*
L. fuscus	*L. marinus*
L. argentatus	*L. fuscus*
L. cachinnans	*L. argentatus + cachinnans*
L. occidentalis + livens	*L. occidentalis + livens*
L. dominicanus	*L. californicus*
L. californicus	Subgroup
L. canus	*L. canus*
L. delawarensis	*L. delawarensis*
L. audouinii	Subgroup
Subgenus *Adelarus*	*L. audouinii*
L. hemprichii	Subgenus *Xema*
L. heermanni	The grey and Heermann's gulls
L. modestus	*L. heermanni*
Subgenus *Ichthyaetus*	*L. modestus*
L. ichthyaetus	The primitive hooded species
Genus *Hydrocoleus*	*L. hemprichii*
Subgenus *Atricilla*	*L. ichthyaetus*
H. atricilla	*L. melanocephalus*
Subgenus *Hydrocoleus*	*L. atricilla*
H. minutus	*L. pipixcan*
H. philadelphia	The masked species
H. genei	*L. philadelphia*
H. ridibundus	*L. genei*
H. bulleri	*L. ridibundus*
H. pipixcan	*L. serranus*
H. melanocephalus	*L. scopulinus*
Subgenus *Cirrhocephala*	*L. cirrocephalus*
H. scopulinus	The little gull
H. serranus	*L. minutus*
H. cirrocephalus	The kittiwakes
Genus *Rissa*	*R. tridactyla*
R. tridactyla	*R. brevirostris*
R. brevirostris	The swallow-tailed and Sabine's gull
Genus *Creagrus*	*C. creagrus*
C. furcatus	*X. sabini*
Genus *Xema*	Subgenus *Pagophila*
X. sabini	*P. eburnea*
Genus *Pagophila*	
P. eburnea	

(1959) noted a strong similarity of behaviour between *L. modestus* and *L. pipixcan*, but was unable to study *L. heermanni*. The main groups of hooded gulls identified by our DNA data correspond to the 'masked' (the *L. genei* – *L. ridibundus* group) and 'primitive hooded' (other hooded species) groups defined by Moynihan (1959) on the basis of behaviour. But Moynihan still maintains its 'masked' and 'primitive hooded' groups as sister taxa on the basis of the shared dark hood, although the vocalizations of these 'primitive hooded' gulls sound more like the calls of the large white-headed species than like the rasping calls of the 'masked' gulls (unpublished observations).

Morphological convergence is not restricted to plumage features, though, as illustrated by the poor results of the analysis of the 117 skeleton characters alone performed by Chu (1998).

Taxonomic implications

Results of mtDNA analysis suggest that the current generic arrangement of the tribe Larini is inappropriate since the genus *Larus* as presently recognized is polyphyletic. Either all species should be placed in the same genus, *Larus* (with the possible exceptions of some of the species we have not analysed), or more genera should be recognized. We tend to favour the last option because we feel it better describes the diversity of the tribe Larini and it introduces fewer changes than the suppression of five well-established genera. We thus propose to keep the following combinations: *Rissa tridactyla*, *R. brevirostris* and *Creagrus furcatus*. We propose to revalidate the genus *Hydrocoleus* Kaup 1829 for the little gull, *H. minutus*. *Rhodostethia* is retained pending further studies for *R. rosea*. The genus *Chroicocephalus* Eyton 1836 is available for *C. genei*, *C. philadelphia*, *C. ridibundus*, *C. serranus*, *C. cirrocephalus* and *C. scopulinus*. The assignment to this group of *C. novaehollandiae*, *C. hartlaubii*, *C. bulleri*, *C. maculipennis* and *C. brunnicephalus* is rather straightforward. *C. saundersi* can be placed provisionally in this genus for the sake of parsimony. We retain *P. eburnea* and *X. sabini* because we feel their morphological, ecological and behavioural differences justify retaining these species in separate genera. The genus *Larus* Linnaeus 1758 would then be restricted to the remaining 31 species, including *L. scoresbii*, *L. pacificus*, *L. belcheri*, *L. atlanticus* and *L. crassirostris* pending further studies to confirm their position.

Evolution of plumage

Our results show that gull plumage has been the subject of deep and fast modifications by natural selection, including several convergent acquisitions of common features. The most striking example is the apparently repeated and independent acquisition by several tropical species (e.g. *L. heermanni*, *L. modestus* and *L. hemprichii*) of a dark plumage, quite unlike the typical pattern of other gulls. These dark species are not related to each other and originate from independent radiations in the tropics from northern clades. This dark body coloration can be straightforwardly interpreted as an adaptation to a common constraint, the dark feathers being made more resistant to sun bleaching by melanin (Van Tyne & Berger, 1971; Bretagnolle, 1993). But other unknown selective forces may act on plumage characters in these species, being

responsible for the remarkably similar pale head, contrasting with the dark body, of adult *L. modestus* and *L. heermanni*.

This evolution toward melanism is mirrored by the white body colour of arctic gulls. *L. hyperboreus*, *L. glaucoides* and *P. eburnea* spend their whole life cycle in or around arctic areas, and the reduction of dark pigments in the plumage, most pronounced in the wholly white *P. eburnea*, is probably a consequence of their common environment. *X. sabini*, which breeds under comparable latitudes but migrates to tropical areas out of breeding season where it winters at sea, has retained a contrasted body plumage.

Our phylogenetic scenario illustrates that the presence of a dark hood has no value for determining species relationships, despite frequent emphasis on this character being put in, for example, Dwight (1925) or Moynihan (1959). Skuas, terns and skimmers (the other members of the Larinae subfamily) all have a dark cap reminiscent of the dark hood of many gulls. This suggests that the dark hood is the ancestral state in gulls. Furthermore, under this hypothesis, the hood would have been lost seven times within our species sample, whereas as many character changes plus several reversions would be required if it were a derived character. Nevertheless, analysis of our data does not permit directionality in the evolution of this character to be unambiguously determined. Chu (1998), using a cladistic approach, came to the conclusion that the dark hood is indeed the ancestral state. A white head has been considered as a possible adaptation for increased fishing efficiency (Isenmann, 1976). Instead of a basal separation of white-headed and dark-headed clades, our results indicate recurrent independent modifications of the head coloration among gulls.

Biogeography and history

Although the phylogenetic arrangement of gulls resulting from our study differs from all previous hypotheses, several species group are retained in our analysis. It thus seems reasonable to take into account some of the species that we did not analyse when discussing the history of these species groups. The species that compose our 'masked' species group (*C. ridibundus* and relatives) were already grouped by Cramp & Simmons (1983) and Moynihan (1959). *C. brunnicephalus*, *C. maculipennis*, *C. bulleri*, *C. hartlaubii* and *C. novaehollandiae*, placed by these authors in the same species group, seem safely attributable to this clade, which shows a fairly homogeneous juvenile plumage across species (unpublished observations). The *L. argentatus* complex *sensu lato* is a homogeneous group identified by all previous studies and by our results. *L. schistisagus, L. armenicus* and *L. glaucescens* are typical members of this group, included in the 'white-headed' species group. *L. relictus* is intermediate in size and plumage characteristics between *L. melanocephalus* and *L. ichthyaetus* and shares with the latter species a white chick plumage (Kitson, 1980). These last two species being members of the same group, *L. relictus* can be placed also in the 'black-headed' species group. *L. leucophthalmus* appears as closely related to *L. hemprichii* ('black-headed' species group) (Grant, 1982). The remaining eight species, *C. saundersi* ('black-headed' species group? (Cramp & Simmons, 1983), *L. fuliginosus* ('hooded' species group? (Moynihan, 1959), *L. belcheri*, *L. atlanticus*, *L. crassirostris*, *L. pacificus*, *L. scoresbii* and *R. rosea*) cannot be attributed presently with certainty to one of the eight groups because of morphological or behavioural peculiarities.

The two groups with the largest number of species ('white-headed' species group: 16 species; 'masked' species group: 11 species) have the widest geographical distribution. The *C. genei* – *C. ridibundus* 'masked' group has representatives in America, Africa, Australia and New Zealand. We suggest that it originated in the Palearctic where three species still live, including *C. genei*, which our results identify as one of the oldest stem within this group (Sibley & Monroe, 1990; del Hoyo *et al*., 1996). The *L. argentatus* complex and its close relatives ('white-headed' species group) occupy the whole northern hemisphere and have one southern hemisphere representative (*L. dominicanus*) which, although it occupies three continents, has little or no subspecific differentiation (del Hoyo *et al*., 1996), possibly indicating a recent colonization.

The other groups seem to have evolved within more restricted areas and show an even clearer origin. The example of the 'black-headed' species group (*L. ichthyaetus*, *L. melanocephalus* and *L. relictus*; *L. hemprichii* and *L. leucophthalmus*; *L. audouinii*) is particularly striking. It is made up of three kinds of morphotypes which strongly differ in plumage, but all species are mainly distributed in the southern/central areas of the Palearctic region, despite an early separation from the other clades. Similarly, the 'hooded' group (*L. pipixcan* and *L. atricilla*; *L. modestus*, and possibly *L. fuliginosus*) is another example of ancient differentiation without long-distance range extension. Similarly, we hypothesize that *P. eburnea* and *X. sabini* probably differentiated in the high arctic rather than having both colonized the arctic after differentiation.

No major geological events able to prevent dispersion by such good flyers as gulls has occurred in the last million years inside the range of the above-mentioned clades. The absence of extrinsic barriers to isolate populations or colonization of distant areas suggests a nearly parapatric mode of speciation for the species concerned. Friesen & Anderson (1997) came to the same conclusions for another group of colonial waterbirds, the Sulidae (gannets and boobies). It is likely that a strong philopatry in these birds compensated for their high dispersal capacities and allowed them to differentiate within flying distance of each other. This process was probably helped

in gulls by their abilities to develop reproductive isolation by rapid evolution of premating mechanisms, as illustrated by the very low genetic and morphological differentiation (indicating very recent speciation) between members of the herring gull species complex.

Whereas several evolutionary events seem strongly established by our DNA sequence data, their dating is clearly more questionable. Our interpretation that all extant gull lineages evolved within the last 6 million years contradicts information from fossil remains attributed to the genus *Larus* (e.g. *L. desnoyersii*, *L. elegans*, *L. totanoides*) as early as 25–30 MYA (Bochenski, 1997), while it fits well the commonly given value of 2% divergence (all substitutions) per million years for animal mtDNA, including birds (Shields & Helm-Bychowski, 1988). Based on this value of mtDNA evolution rate, the maximum divergence time among Larini would be around 5 million years. Reconciling fossil records and DNA data would require an extremely slow evolution rate for both nuclear and mitochondrial DNA (less than 0.5% divergence per million years for mitochondrial DNA). This calls for a re-examination of the fossil bones that could in fact belong to the Larinae (*sensu* Sibley & Ahlquist, 1990) rather than *Larus* in the strict sense. The absence of recorded fossil Stercorariini, Rynchopini or Sternini from the same time (Bochenski, 1997) argues in favour of this interpretation. Indeed, *L. desnoyersii* may be a fossil Stercorariini (Olson, 1985) whereas *L. totanoides* and *L. elegans* combine some characters of modern Stercorariini and Sternini with typical Larini characters (Milne-Edwards, 1867) and are not true gulls (C. Mourer-Chauviré, personal communication).

For a related group, the auks (subfamily Alcinae), Friesen *et al*. (1996) failed to resolve the deepest node of their phylogenetic tree, which also showed a rapid initial cladogenesis followed by more recent speciation events. The same pattern of quick divergence of the main species groups, resulting in unresolved relationships among them, also occurred in the warblers of the *Phylloscopus* genus (Richman & Price, 1992). Although not a general tendency, this recurrent finding in birds of phylogenetic trees combining well-defined species group with poorly supported nodes inside and between these species groups suggests that these uncertainties could be mainly due to particular speciation processes. If so, the lack of resolution of both the ancient nodes and of the species relationships inside the species groups would be explained by an alternating of intense initial diversification giving rise to several lineages at the same time, followed by periods of independent evolution of these lineages. Uncertainty about basal relationships within Larini may thus be the result of a quick differentiation of the main lineages in a relatively short time rather than of a loss of phylogenetic information through saturation of the nucleotide sites. If so, further sequencing may not improve dramatically the resolution level between clades.

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