The Auk 119(3):603–620, 2002

SYSTEMATICS OF LARGE WHITE-HEADED GULLS: PATTERNS OF MITOCHONDRIAL DNA VARIATION IN WESTERN EUROPEAN TAXA

PIERRE-ANDRÉ CROCHET,[1,2,3,4] JEAN-DOMINIQUE LEBRETON,[1] AND FRANÇOIS BONHOMME[2]

[1]*CEFE-CNRS, 1919 route de Mende, F-34293 Montpellier cedex 05, France;*

[2]*Laboratoire Génome, Populations, Interactions, CNRS UMR 5000—Université de Montpellier 2, Place E. Bataillon, F-34095 Montpellier cedex 5, France; and*

[3]*Station Biologique de la Tour du Valat, Le Sambuc, F-13200 Arles, France*

ABSTRACT.—Although the large white-headed gull group (genus *Larus*) has long been a model in speciation studies, the systematic status and evolutionary relationships of many of its taxa remain unresolved. We used mitochondrial DNA control region and cytochrome-*b* gene sequences in an attempt to resolve some of those uncertainties. In contrast to previously published results based on nuclear markers, mitochondrial DNA was found to be strongly structured among species, indicating that mitochondrial gene flow is very low. Phylogenetic relationships remain largely unresolved, mainly because of the low amount of variation between species. Horizontal transfer of mitochondrial lineages is demonstrated or suspected between most taxa and obscured the reconstruction of the history of the group. The Mediterranean form *michahellis* was as differentiated from the other western European species as these are from each others, confirming it is neither conspecific with *L. fuscus* nor with *L. argentatus*. The forms *fuscus* and *graellsii* do not show any significant differences in haplotypes frequencies, arguing for their subspecific status. *Received 27 September 2000, accepted 30 January 2002.*

RÉSUMÉ.—Bien que le groupe des goélands (genre *Larus*) est depuis longtemps un modèle utilisé pour les études sur la spéciation, le statut systématique et les relations évolutives de la plupart de leurs taxa restent non résolu. Nous avons utilisé des régions contrôles d'ADN mitochondrial et des séquences du gène cytochrome-*b* pour tenter de résoudre certaines de ces incertitudes. À la différence des résultats basés sur des marqueurs nucléaires précédemment publiés, nous avons trouvé que l'ADN mitochondrial était fortement structuré parmi les espèces, indiquant que le flux de gène mitochondrial est très faible. Les relations phylogénétiques restent en grande partie non résolu, principalement en raison de la faible quantité de variation entre espèces. Le transfert horizontal de lignées mitochondriales est démontré ou suspecté entre la plupart des taxa et obscurcit la reconstitution de l'histoire du groupe. La forme méditerranéenne *michahellis* était aussi différenciée des autres espèces d'Europe de l'ouest que ces dernières le sont les unes par rapport aux autres, confirmant l'absence de conspécificité avec *L. fuscus* et *L. argentatus*. Les formes *fuscus* et *graellsii* ne montrent aucune différence significative dans les fréquences d'haplotypes, argumentant dans le sens de leur statut sous-spécifique.

THE LARGE WHITE-HEADED GULLS (i.e. species more or less closely related to *Larus argentatus*; see below for a more precise definition of the group) have always represented a notorious systematic challenge (see Table 1; del Hoyo et al. 1996). The main reasons for that are the frequency of hybridization occurring even between well-established species (e.g. see Hoffman et al. 1978, Pierotti 1987, Bell 1996), the strong plumage similarity between some taxa (see Grant 1986) even when they seem to be reproductively isolated (e.g. Klein and Buchheim 1997), and the high level of phenotypic differentiation achieved in some cases without apparent reproductive isolation. Furthermore, many taxa of contentious status live in the Arctic regions of North America or within the limits of the former Soviet Union, making field as-

[4] Present address: Laboratoire de Biogéographie et Ecologie des Vertébrés, Ecole Pratique des Hautes Etudes, Université Montpellier II, c.c. 94, 34095 Montpellier cedex 5, France. E-mail: crochet@univ-montp2.fr

TABLE 1. Current classification of the *argentatus–fuscus* complex and suggested changes (not based on our results).

Name	del Hoyo et al. 1996	Approximate range	Suggested systematic changes previously published and remarks
marinus Linnaeus, 1758	*L. marinus*	West Europe, east North America	None.
fuscus Linnaeus, 1758	*L. fuscus fuscus*	From the Baltic and north Norway coasts to the White Sea	None.
intermedius Schiøler, 1922	*L. fuscus intermedius*	Netherlands to south Norway	*L. graellsii intermedius*. See *graellsii* below.
graellsii Brehm, 1857	*L. fuscus graellsii*	Iceland, British Isles, France, locally north Spain	*L. graellsii graellsii*. Based on differences in phenotype, behavior and feeding ecology between *fuscus* and *graellsii* and *intermedius*, some authors proposed to treat them as distinct species (see Sangster et al. 1999), although morphological variation in *fuscus* and especially *intermedius* makes some birds indistinguishable (Jonsson 1998b, L. Jonsson pers. comm.). Our genetic results do not support this change.
heuglini Bree, 1876	*L. fuscus heuglini*	North Russia, from Kola Peninsula to west of the Yenisey River	*L. heuglini heuglini*. Filchagov (1994) claimed that *heuglini* is reproductively isolated from *argentatus* and *fuscus* along the White Sea coasts. Liebers et al. (2001) showed that this form is closely related to *fuscus*.
taimyrensis Buturlin, 1913	Synonym of *heuglini*	Lower Yenisey Valley and southwestern Taimyr Peninsula	*L. heuglini taimyrensis*. Further work needed to assess validity of this taxon.
argentatus Pontoppidan, 1763	*L. argentatus argentatus*	Europe from Denmark to east Kola Peninsula	None.
argenteus Brehm, 1822	*L. argentatus argenteus*	West Europe from Iceland to northwest Germany	None.
smithsonianus Coues, 1862	*L. argentatus smithsonianus*	North America	*L. smithsonianus*. See text. Certainly not a member of the Eurasian LWHG clade.
vegae Palmén, 1887	*L. argentatus vegae*	North Siberia from Taimyr eastward (western part of the range: *birulai*?)	*L. vegae vegae*. Study of museum specimens reveal very few specimens intermediate between *vegae* and *taimyrensis* (Filchagov 1994, L. Jonsson pers. comm., P. Yésou pers. comm.), suggesting reproductive isolation. Contra for example Haffer (1982), Filchagov et al. (1992), del Hoyo et al. (1996), most of Taimyr peninsula seem to be inhabited by *vegae* or *birulai* (depending whether one recognizes this last taxon or not) (Kennerley et al. 1995, L. Jonsson pers. comm., P. Yésou pers. comm.).
birulai Pleske, 1928	Synonym of *vegae*	Western part of the range of *vegae*	*L. vegae birulai*. Further work needed to assess validity of this taxon.

TABLE 1. Continued.

Name	del Hoyo et al. 1996	Approximate range	Suggested systematic changes previously published and remarks
cachinnans Pallas, 1811	*L. cachinnans cachinnans*	Black Sea, Caspian Sea, and east to the Lake Balkash	None.
mongolicus Sushkin, 1925	*L. cachinnans mongolicus*	From east of the Altai Mountains to Mongolia	None. Genetic data suggest it is not related to *cachinnans* (Liebers et al. 2001).
barabensis Johansen, 1960	*L. cachinnans barabensis*	Steppes of north Kazakhstan	None. Probably closely related to *heuglini* rather than to *cachinnans* (Liebers et al. 2001, L. Jonsson pers. comm.)
michahellis Naumann, 1840	*L. cachinnans michahellis*	Mediterranean Sea, Atlantic coasts of Iberia, France, and Morocco	*L. michachellis michahellis*. See text.
atlantis Dwight, 1922	*L. cachinnans atlantis*	Canaries, Madera, Azores (as traditionally given. See remarks.)	*L. michahellis atlantic*. Beaubrun (1988) and Dubois (2001) considered *atlantis* to be limited to the Azores, with the populations from Madera, Canaries and southern Morocco intermediate between nominate subspecies and *atlantis*.
armenicus Buturlin, 1934	*L. armenicus*	Anatolia and Caucasus region	None. See Liebers and Helbig (1999) and Liebers et al. (2001) for range and relationships with *michahellis, cachinnans* and *barabensis*

sessment of their level of reproductive isolation difficult.

A recent molecular phylogeny of the Larini (Crochet et al. 2000) indicated that the traditionally recognized white-headed species group (Dwight 1925, ''subgenus *Larus*''; Moynihan 1959, ''white-headed species group'') constitutes a monophyletic assemblage. The only changes from traditional views on the composition of that group were the unambiguous exclusion of Audouin's Gull (*L. audouinii*) from the white-headed species and the identification of the Heermann's Gull (*L. heermanni*) as the most basal species in that group. The Mew Gull (*L. canus*) and Ring-billed Gull (*L. delawarensis*) were found to be the next species to have diverged in that group. The remaining species constitute the large white-headed gulls (see Crochet et al. 2000 for the composition of the large white-headed species group). Within the large white-headed gulls, two species were placed in a basal position with a strong support from the molecular data: the Western Gull (*L. occidentalis*) and Yellow-footed Gull (*L. livens*). The remaining species constitute the *fuscus* clade, which includes most of the taxa of contentious systematic status. The relationships within the *fuscus* clade were not discussed in Crochet et al. (2000), and the aim of this article is to use our molecular data to discuss the evolution and systematics of those members of the *fuscus* clade for which we could obtain data on mitochondrial polymorphism.

Few previous studies have tried to clarify relationships within the *fuscus* clade with the help of molecular markers, in spite of the growing use of those methods in avian systematics. The lack of differentiation observed with enzymatic markers even between well-established species (Snell 1991a) left little hope that allozymes could help solve the problem. Sequencing of mitochondrial DNA seemed more promising, because a preliminary study based on a small number of specimens found different haplotypes in a short cytochrome-*b* segment between *L. argentatus*, *L. fuscus*, and *L. michahellis* (Wink et al. 1994). Liebers et al. (2001) used hypervariable control region I (HVR-I) to elucidate relationships among several taxa classified in *L. fuscus*, *L. armenicus*, and *L. cachin-*

nans by del Hoyo et al. (1996; see Table 1). Although those authors identified several cases of lineage sharing that they attribute to introgression, they did not precisely analyze variation in haplotypes frequencies.

In the present study, we use variation in mitochondrial control region and cytochrome-*b* haplotypes to investigate levels of mitochondrial gene flow and reproductive isolation between various members of the large white-headed group, focusing on the *argentatus–fuscus* complex. We first present additional results on phylogenetic relationships among large gulls based on sequencing of the cytochrome-*b* and control region segments. Considering these, the systematic status of the North American taxon *smithsonianus* is discussed. We then use frequencies of cytochrome-*b* haplotypes to study degree of reproductive isolation between the sympatric *L. marinus*, *L. argentatus*, and *L. fuscus* in western Europe, and with the Arctic species *L. hyperboreus*. Those species raise no systematic questions, but provide levels of haplotype sharing among undisputed species. Patterns of differentiation between those unquestioned species are then used to evaluate the relationships of *L. michahellis* with the other western European taxa. Geographical variation and recent history of *L. fuscus* and *L. argentatus* is discussed in relation to our genetic results. Some information is also provided on the poorly known Siberian taxa. We made every effort to interpret genetic data with respect to what is known on the morphological variation and reproductive isolation of the taxa studied.

An overview of the taxonomic situation of the members of the *argentatus–fuscus* complex, including proposed splits and resulting classification of all taxa, is given in Table 1. We follow the classification of del Hoyo et al. (1996) except for *michahellis* that we treat as a distinct species (see below). We use a single Latin name to designate valid basal taxa (subspecies or monotypic species), whereas a binomen designates a species. Thus, *argentatus* means *L. argentatus argentatus*, but *L. argentatus* means the Herring Gull, comprising several subspecies. We tried as much as possible to use basal taxa names, but that was not always possible or desirable.

METHODS

Samples origin.—The list of sampled taxa is given in Table 2, with the samples' localities and collector's name. Most samples were of breeding adults or embryos or nonflying chicks, but some specimens from Finland were caught at refuse dumps. Sampling siblings was avoided by taking only one embryo per clutch or one chick by family. Taxon determination problems were usually few, but some *graellsii* and *argenteus* samples from mixed colonies in western France are feathers plucked from chicks which may be difficult to differentiate (see below). One specimen of *heuglini* was an immature bird caught at a refuse dump in Finland, outside the main range of that taxon, but recent evidence suggests that *heuglini* occurs regularly in Finland (Eskelin and Pursiainen 1988; Rauste 1999a, b). For *smithsonianus*, the nine specimens used in this study were only sequenceed for the cytochrome-*b* gene. The position of *smithsonianus* in the phylogenetic tree was thus determined using cytochrome-*b* and control region sequences of a specimen from Québec (see Table 2) sequenced by J.-M. Pons (pers. comm.). That specimen had the same cytochrome-*b* haplotype as our nine individuals from Manitoba.

Extraction, amplification, and sequencing.—Available samples consisted of muscle in ethanol, dried or ethanol-preserved feather bases, blood in buffer or in ethanol, or tissue from dried wings. DNA from muscles and feather bases was extracted by a complete digestion in 400 μL of 5% Chelex 100 (Biorad, Hercules, California) with 1 mg mL^{-1} proteinase K followed by a 10 min boiling. Extractions from blood and dried wings were performed using Qiaamp tissue extraction kit (Qiagen, Santa Clarita, California) following the supplier's procedure. Polymerase chain reaction (PCR) amplifications were carried out in 50 μL volumes containing 1× amplification buffer per 1 unit of *Taq* DNA polymerase, 1.5 mmol $MgCl_2$, 0.2 mmol of each dNTP, and 0.4 μmol of each primer. Direct sequencing of one strand was performed on an automated sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) following the supplier's procedures.

A 280 to 300 bp long segment from the cytochrome-*b* gene (starting around position 15015 in the chicken [*Gallus gallus domesticus*] mitochondrial DNA sequence; Desjardins and Morais 1990) was sequenced from all specimens listed in Table 2. In addition, a 640 to 650 bp long segment of the control region, usually starting around position 465 of the chicken sequence and ending at the heteroplasmic control repeats (see Berg et al. 1995), was sequenced from 12 *michahellis*, 8 *argenteus*, 2 *marinus*, 2 *graellsii*, 2 *L. dominicanus*, 2 *L. hyperboreus*, 1 *glaucoides*, 1 *kumlieni*, 2 *L. schistisagus*, 1 *L. californicus*, and 1 *thayeri*. We used as an outgroup *L. crassirostris* Vieillot, 1818 which is closely related to (but not a member of) the typical large white-headed species (P.-A. Crochet unpubl. data).

The amplification primers for the control region were L438 (5′-TCACGTGAAATCAGCAACCC-3′)

TABLE 2. List of taxa used, origin of specimens, and voucher specimen (if any).

Taxon	Locality (number of specimens)	Type of specimens	Collector / sample origin	Voucher specimen
crassirostris	Teuri Island, Hokkaido, Japan	Nonflying chicks	C. Michiyo	No
livens	San Pedro Martir Island, Mexico (2)	?	B. Tershy, from R. Bradbury	No
occidentalis	Grays Harbor, Washington (1)	?	Louisiana State University Museum	LSUMZ B-20480
thayeri	Cameron Parish, Louisiana (1)	?	Louisiana State University Museum	LSUMZ B-21816
glaucoides	Nolsoy, Faeroe Islands (1)	?	Zoological Institute of the University of Copenhagen	?
kumlieni	Coats Island, Northwest Territory, Canada (1)	?	T. Gaston, from R. Bradbury	No
schistisagus	Teuri Island, Hokkaido, Japan (2)	Nonflying chicks	C. Michiyo	No
hyperboreus	Taimyr Peninsula, Russia (2)	Adults	P. Mortensen / Swedish Museum of Natural History	NRM 946577 / 946581
hyperboreus	Coats Island, Northwest Territory, Canada (10)	?	T. Gaston, from R. Bradbury	No
californicus	Mono Lake, California (2)	?	J. Jehl, from R. Bradbury	No
dominicanus	Kerguelen Islands (1)	Adult	Paris National History Museum	MNHN 1951-668
dominicanus	South Island, New Zealand (1)	Adult	M. Renner	No
marinus	Béniguet Island, Brittany, France (2)	Nonflying chicks	P. Yésou and D. Mourier	No
marinus	Turku, Finland (1)	Adult	A. Forsten and V. Rauste	No
marinus	Finnmark, Norway (2)	?	R. Barret, from R. Bradbury	No
marinus	Helgoland, Germany (1)	?	Louisiana State University Museum	LSUMZ B-27027
michahellis	Isla de Ons, Pontevedra, Spain (6)	Breeding adults	A. Velando	No
michahellis	Berlenga Islands, Portugal (8)	Breeding adults	L. Morais	No
michahellis	Camargue, France (1)	Breeding adult	P.-A. Crochet	No
michahellis	Cassa di Colmata, Mira, Italia (4)	Nonflying chicks	G. Cherubini	No
michahellis	Essaouira, Morocco (10)	Embryos	P.-A. Crochet	No
mongolicus	Lake Baïkal, Russia (2)	Breeding adults	S. Pyzhianov and P. Yésou	Wings in P.Y. collection
barabensis	Near Omsk, Russia (3)	Breeding adults	A. Filchagov, from P. Yésou	Wings in P.Y. collection
vegae (*birulai*)	Taimyra Estuary, north Taimyr, Russia (3)	Adults	A. Filchagov, from P. Yésou	Wings in P.Y. collection

TABLE 2. Continued.

Taxon	Locality (number of specimens)	Type of specimens	Collector / sample origin	Voucher specimen
vegae (*birulai*)	Polar Station Anderya, Taimyr, Russia (1)	Adult	P. Mortensen, Swedish Museum of Natural History	NRM 946571
vegae (*birulai*)	Chelyuskin Peninsula, Taimyr, Russia (2)	Adult and chick	P. Mortensen, Swedish Museum of Natural History	NRM 946585 / 946582
heuglini	Kuopio, Finland (1)	Immature	R. Juvaste	Photo
heuglini	Kanin Peninsula, Russia (2)	Adults	P. Mortensen, Swedish Museum of Natural History	NRM 946646 / 946650
heuglini	West Yamal Peninsula, Russia (1)	Adult	P. Mortensen, Swedish Museum of Natural History	NRM 946613
argentatus	Turku, Finland (10)	Nonbreeding adults	A. Forsten, V. Rauste and A. Lindholm	No
argentatus	Vardo, Finnmark, Norway (8)	?	R. Barret, from R. Bradbury	No
argenteus	Béniguet Island, Brittany, France (18)	Nonflying chicks	D. Santer, D. Mourier and P. Yésou	No
graellsii	Béniguet Island, Brittany, France (14)	Nonflying chicks	D. Santer, D. Mourier and P. Yésou	No
graellsii	Great Saltee Lake, Ireland (6)	?	O. Merne, from R. Bradbury	No
fuscus	Kuopio, Tuusniemi, Outokumpu, Kontiolahti, Vaasa, Oravainen, Kokkola, all Finland (18)	Breeding adults and nonflying chicks	R. Juvaste	No
smithsonianus	Manitoba, Canada (9)	?	R. Evans and G. Fox, from R. Bradbury	No
smithsonianus	Saintes Maries Islands, Québec, Canada (1)	Nonflying Chicks	G. Capdelaine and J.-F. Rail	No

(Wenink et al. 1993) and H1248 (5′-CATCTTCAGTGCCATGCTTT-3′). Sequencing primers were L438, L699 (5′-ATAAACCCCTCCAGTGCACC-3′) and L892 (5′-GTGTAGTGCTCAATGGACATG-3′). A 280 bp segment of the cytochrome-*b* gene was amplified and sequenced using primers L15008 (5′-AACTTCGGATCTCTACTAGG-3′) and H15326 (5′-GAATAAGTTGGTGATGACTG-3′). ''L'' refers to light and ''H'' refers to heavy strands, and the numbers refer to the position of the 3′ nucleotide of the primer in the White Leghorn Chicken (*Gallus gallus*) mitochondrial DNA sequence (Desjardins and Morais 1990).

Data analyses.—To determine the evolutionary history of the typical large white-headed gulls, phylogenetic analyses were performed on composite haplotypes including the cytochrome-*b* and control region segments. Phylogenetic trees were reconstructed using the maximum-likelihood method (DNAML) in the PHYLIP package, version 3.57c (Felsenstein 1993) and the neighbor-joining method using distance matrix based on the Kimura two-parameter model with MEGA version 2.1 (Kumar et al. 2001). We computed the likelihood of the maximum-likelihood tree obtained with the default setting for several integer values of the transitions–transver-

TABLE 3. Sequence of the haplotypes observed in the *fuscus* clade (variable sites only) and their name (in bold).

	Control region	cytochrome-*b*	CR	CTB
MIC (*michahellis*)	ATGTGATTTACA	GGTTATCATATC	AF268527	AF268493
MAR (*marinus*)	.....	.A.....	AF268529	AF268496
ARG (*L. argentatus*)	.....	AACC....C...	AF268530	AF268495
70	????????????	.A...C.....		AF444253
HEU (*heuglini*)	????????????	.ACC....C...		AF444254
FUS2	?.....	.A.C..T.CG.?	AF444255	AF444256
FUS (*fuscus*)	.....	.A.C....CG..	AF268531	AF268494
SMI (*smithsonianus*)	......C.C...	.A.CG...C..T	AF444266	AF444257
***dominicanus*1**[a]	...C.....	.A.C....C.C.	AF268536	AF268497
***dominicanus*40**[a]	...C.....G..	.A.C....C.C.	AF444258	AF444259
g. glaucoides	.....C.T.	.A.CG..GC...	AF268533	AF268499
g. kumlieni	.....C.T.	.A.CG...C...	AF444260	AF444261
thayeri	GCT.T...C.T.	.A.CG...C...	AF268534	AF268498
HYP (*L. hyperboreus*)	G.....C.T.	.A.CG...C...	AF268535	AF268500
***schistisagus*1**	.....G..C.T.	.A.CG...C...	AF444262	AF444263
***schistisagus*2**	.....C.T.	.A.CG...C...	AF444264	AF444265
californicus	...C...CC...	.A.CG...C...	AF268532	AF268503

Three-letter names designate widespread haplotypes, whereas longer names designate haplotypes found in one individual. A dot indicates a base identical to the first sequence, ''?'' indicates undetermined base. GenBank accession numbers are given for the control region (CR) and cytochrome-*b* (CTB) sequences.

[a] dominicanus1 = *Larus dominicanus* from New Zealand, dominicanus40 = *Larus dominicanus* from Kerguelen Islands (see Table 2).

sions ratio ranging from 2 to 10. The likelihood was maximal when this ratio was equal to 5, and we used that value to re-estimate the maximum-likelihood tree (Felsenstein 1993). We evaluated the robustness of the nodes that were identified by both maximum-likelihood and neighbor-joining methods by bootstrap (Felsenstein 1985) based on (for the sake of computational rapidity) the neighbor-joining method only, using 1,000 replications.

Because most members of the *argentatus–fuscus* complex and *L. marinus* were found to have mostly the same control region sequences (see Table 3 and Crochet and Desmarais 2000), only the cytochrome-*b* segment was sequenced in all our samples of those taxa. Haplotype identification is thus based only on cytochrome-*b* sequences for most of those specimens. Additionally, parts of the control region were sequenced for some of the samples to check haplotype identification (for the *L. hyperboreus* haplotype for example, because in that case several substitutions in the control region separate it from the common *argentatus–fuscus* haplotype).

Genetic differentiation between the populations (here, the taxa) was evaluated using an F_{ST} approach. Values of F_{ST} were estimated by the parameter θ (Weir and Cockerham 1984) using the GENETIX 4.01 software (Belkhir et al. 1998). The significance of the θ values was evaluated by comparing observed values to distribution of values obtained from 1,000 random permutations of the individuals between the different populations. That approach is based only on differences in haplotype frequencies between the populations. It does not take into account the relationships between the haplotypes. Other methods are available to take account of that information. One of those is the analysis of molecular variance (AMOVA; Excoffier et al. 1992). We also performed AMOVA, but the results were not qualitatively different from those based on F_{ST} and will not be detailed here. As a rule, taking into account relationships between haplotypes is important when many haplotypes falling into well-supported clades are present in the population. In our case, the relationships between haplotypes are poorly determined and there is a small number of haplotypes.

RESULTS

Genetic variation, haplotypes definition.—Sequencing of the domain II and domain III of the control region revealed that those regions are extremely conserved among the large white-headed species, with *L. marinus*, *L. fuscus*, *L. michahellis*, and *L. argentatus* having identical sequences (Crochet and Desmarais 2000). A number of haplotypes are thus identified by their cytochrome-*b* sequence only. The sequence, name, and GenBank accession numbers of the haplotypes identified are given in Table 3 (see Crochet et al. 2000 and Crochet and Desmarais 2000 for details on the sequences obtained) except for *L. crassirostris* (GenBank accession number AF444267 for the control region and AF444268 for the cytochrome-*b*).

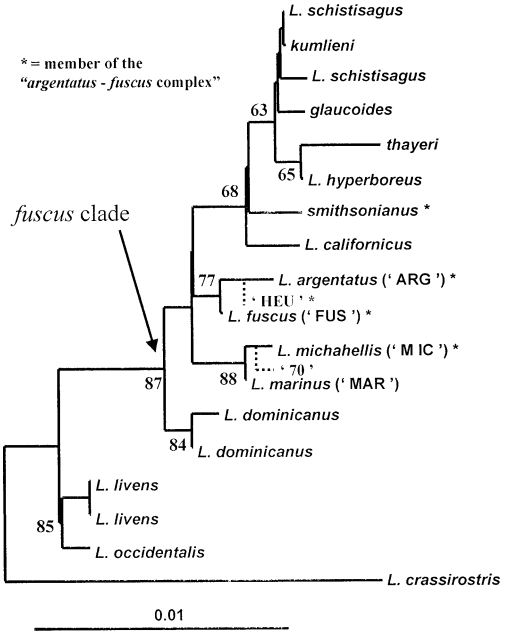

FIG. 1. Maximum-likelihood tree of the large white-headed gulls based on cytochrome-*b* and control region sequences (see text). Values at the nodes indicate bootstrap support for the nodes also identified by the neighbor-joining method based on Kimura two-parameter distances, as determined by 1,000 replications under the neighbor-joining method. When only one haplotype was used per taxon, it was the most common one.

The haplotypes were named after the taxon in which they are most frequent to facilitate reference to them. They are usually not diagnostic of that taxon. Two haplotypes (HYP and SMI) differed in the cytochrome-*b* segment by only one substitution situated near the sequencing primer, in a region that is not available for all specimens. That led to an ambiguity for one specimen of *vegae*, which could have either haplotype. Because haplotype SMI was identified only in North America, although haplotype HYP is common among eastern Siberian gulls, we attributed the haplotype HYP to that specimen. No other ambiguous case occurred.

Phylogeny of the large white-headed species.—Figure 1 presents the hypothetical relationships between the various large gull taxa for which we could obtain complete control region and cytochrome-*b* sequences (maximum-likelihood tree with node support values obtained by bootstrap using the neighbor-joining method). When more than one haplotype was found in a given taxon, we usually used the most common one to represent that taxon. Because only two specimens were available for both *L. dominicanus* and *L. schistisagus*, all haplotypes were included in the phylogeny for those species.

Among the LWHG, *L. livens* and *L. occidentalis* were found to have diverged first by all analyses (note that *L. glaucescens* was not available for analysis). The high bootstrap value (95) at the node uniting the remaining LWHG species indicates that this conclusion is strongly supported by the molecular data. The clade comprising the remaining LWHG species (*L. dominicanus*, the ''*argentatus–fuscus*'' complex, and the Arctic species) will be designated as the *fuscus* clade hereafter. The relative position of *L. livens* and *L. occidentalis* (sister species or not) is not clearly determined, but most analyses favor the hypothesis shown in Figure 1. Similarly, the position of *L. dominicanus* as the sister species to all other members of the *fuscus* clade is poorly supported and is not favored by all analyses. Within the *fuscus* clade, the amount of divergence (Kimura two-parameter distance, calculated on the cytochrome-*b* and the control region together) was small and varied from 0.11% between *marinus* and *michahellis* to 0.99% between *thayeri* and *argenteus*, *marinus* or *L. dominicanus*.

The Arctic species (*L. hyperboreus*, *L. glaucoides*, and *L. thayeri*) form a clade supported by a rather high bootstrap value. That clade includes both our specimens of *L. schistisagus*, one of them having the same haplotype as the *kumlieni* specimen. Our *thayeri* specimen branches closer to our *L. hyperboreus* specimen than to either *kumlieni* or *glaucoides*. The *L. californicus* and *smithsonianus* haplotypes are more closely related to these Arctic species than to the Eurasian members of the *fuscus* clade in this tree, but this is not strongly supported (bootstrap value of 63, see Fig. 1).

Based on molecular data, there is no indication that *marinus* is more distantly related to the members of the *argentatus–fuscus* complex than they are to each other. The relationships between the North American–Arctic clade and the members of the *argentatus–fuscus* complex except *smithsonianus* are unclear, but the tree presented in Figure 1 does not depict them as reciprocally monophyletic groups. Maximum-

TABLE 4. Frequency of the cytochrome-*b* haplotypes in the taxa of the *argentatus–fuscus* complex and *L. marinus*.

	argentatus	*argenteus*	*barabensis*	*fuscus*	*graellsii*	*heuglini*	*marinus*	*michahellis*	*smithsonianus*	*vegae*	*mongolicus*
n	18	18	3	18	20	4	6	29	9	6	2
FUS	0.06	0.00	0.33	0.94	0.80	0.25	0.00	0.03	0.00	0.50	0.00
FUS2	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
HEU	0.00	0.00	0.33	0.06	0.05	0.50	0.00	0.00	0.00	0.00	0.00
ARG	0.33	0.78	0.33	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
HYP	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.33	1.00
SMI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
MAR	0.22	0.22	0.00	0.00	0.00	0.00	1.00	0.17	0.00	0.00	0.00
MIC	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.00	0.17	0.00
70	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

likelihood method favored the hypothesis that *L. dominicanus* occupies a basal position relative to other gulls in the *fuscus* clade (Fig. 1), but neighbor-joining placed it within the remaining species (results not shown).

Haplotype distribution.—The frequency of the cytochrome-*b* haplotypes in the taxa of the *argentatus–fuscus* complex is given in Table 4 and Figure 2, and pairwise F_{ST} values in Table 5. The three sympatric species *L. marinus*, *L. argentatus*, and *L. fuscus*, and the parapatric taxon *michahellis* have highly different haplotypes frequencies in Europe (see Tables 4 and 5 and Fig. 2) but there is no fixed differences between them.

We had only six *L. marinus*, so we may not have sampled the polymorphism within that species representatively. All our specimens have the same haplotype (MAR), and because they originate from localities spread over the range of the species, haplotype MAR is certainly a typical *marinus* haplotype. That haplotype is also present in *argenteus* from Brittany and *argentatus* from Scandinavia and in *michahellis* specimens from Essaouira (southern Morocco). We did not find any shared haplotype between *L. marinus* and *L. fuscus*.

The conspecific *argenteus* and *argentatus* differ substantially in haplotype frequencies, *argentatus* having a higher genetic diversity. Our samples of *argentatus* from Finland and Finmark (northern Norway) are characterized by a mixture of the haplotypes MAR and ARG (as in *argenteus* from Brittany) and mainly of the haplotype 70, which is closely related to the MIC haplotype. In addition, the MIC haplotype is found in very low frequency in *L. argentatus* (one bird from Finland out of 36 specimens, see Table 4). We did not find any FUS haplotypes in *L. argentatus argenteus* from Brittany, Western France ($n = 18$), but one FUS haplotype was found in *L. argentatus argentatus* from Eastern Scandinavia ($n = 18$).

The two taxa *graellsii* and *fuscus* share the same main haplotype (FUS) and have extremely similar haplotype frequencies that exhibit no significant departure from the panmixia hypothesis (non significant F_{ST} value). Two ARG haplotypes where found in *L. fuscus graellsii* ($n = 20$) from Brittany and Ireland (in two birds from Brittany). In addition, one HEU haplotype was detected in both *graellsii* and *fuscus* ($n = 18$).

Most *michahellis* have the same MIC haplotype. No other haplotypes were detected in birds from the Mediterranean area, but in birds from the Essaouira colony, both FUS and MAR haplotypes were detected in low frequency (Fig. 2 and Table 4).

The Siberian taxa *heuglini*, *vegae*, *barabensis*, and *mongolicus* are characterized by a lack of original genetic material. Only *heuglini* and *barabensis* have an haplotype (HEU) which is not widespread in other taxa, but this HEU haplotype is never the most common one (although very small sample size precludes proper frequency estimation).

Our sample of *L. hyperboreus* includes two specimens from Russia and 10 specimens from Canada (see Table 2). For the two Russian specimens, we sequenced the cytochrome-*b* and control region segments. For the 10 Canadian specimens, we only sequenced the cytochrome-*b* and the end of the control region because the remaining part of the control region was identical to the corresponding segment in the *ar-*

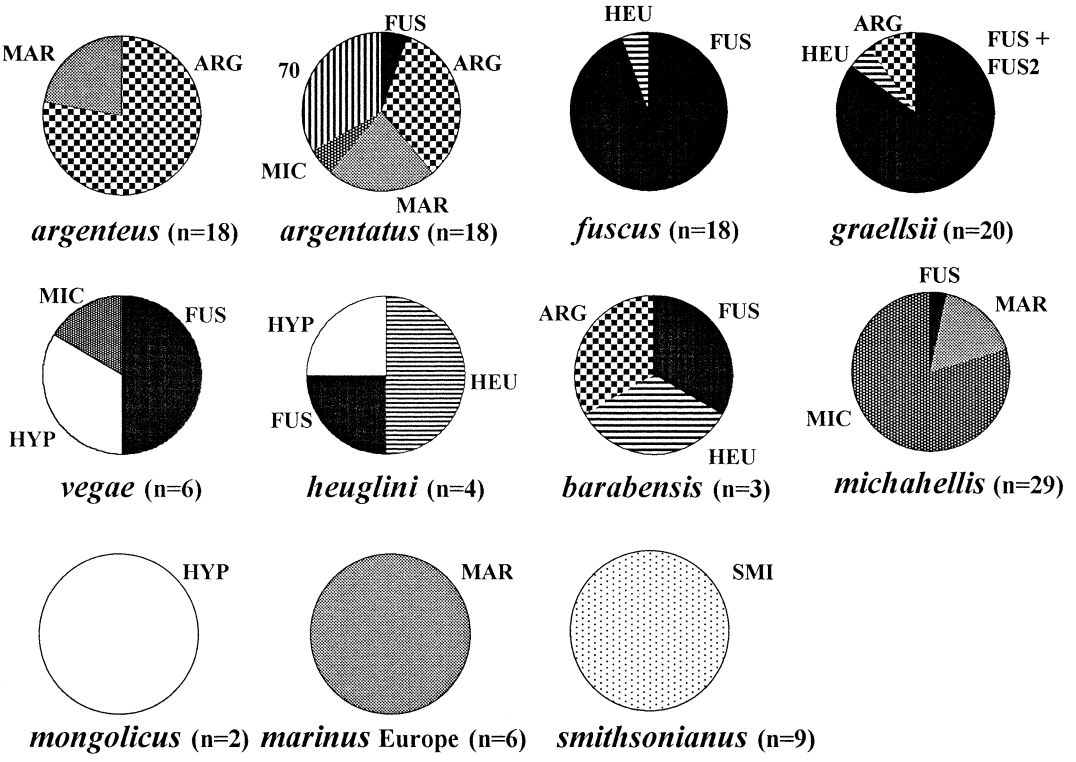

FIG. 2. Frequency of the cytochrome-*b* haplotypes in the taxa of the *argentatus–fuscus* complex. The sample size for each taxon is given after the taxon name.

gentatus–fuscus complex. Whereas 11 specimens have the same (presumable *hyperboreus*) haplotype, one individual from Russia had a control region sequence of the *argentatus–fuscus* type and a cytochrome-*b* sequence identical to the haplotype 70 (only found in *argentatus*, see Fig. 2 and Table 4). Within taxa of the *argentatus–fuscus* complex, *L. hyperboreus* haplotypes have been found in one *heuglini* (out of four), two *mongolicus* (out of two), and two *vegae* (out of six) (see Table 4 and Fig. 2) .

DISCUSSION

Specimen identification.—One striking result of this study is the frequency of shared haplotypes among taxa. It is therefore crucial to interpret those data so that our specimens are representative of those taxa. Sampling has always been done by experienced ornithologists, mostly gull students, often in the course of culling or banding operations. Foreign haplotypes detected in *michahellis* where found in embryos

TABLE 5. F_{ST} values between some taxa of the *argentatus–fuscus* complex based on haplotype frequencies. Only taxa with sufficient sample size were used. Values significant at the 0.05 level are in bold.

	argenteus	*fuscus*	*graellsii*	*michahellis*	*smithsonianus*
argentatus	**0.18225**	**0.53784**	**0.39403**	**0.41586**	**0.53386**
argenteus		**0.76144**	**0.60472**	**0.62774**	**0.76416**
fuscus			0.01538	**0.73737**	**0.92569**
graellsii				**0.63288**	**0.75947**
michahellis					**0.74727**

taken on a breeding colony (Essaouira, southern Morocco) exclusively made of *michahellis*. Furthermore, no other large gull taxon breeds close to that area. Those samples are thus indisputably representative of *michahellis*. All *fuscus* samples were collected on breeding colonies during banding operations. The other species breeding in Finland (*marinus* and *argentatus*) are clearly distinct phenotypically. There is thus no risk of misidentification for *fuscus* samples. Foreign haplotypes were also detected in *graellsii* in western France. Because the foreign haplotypes were typical *argenteus* haplotypes, and because the *graellsii* samples were collected on chicks from mixed colonies with *argenteus*, misidentification might have been suspected. However, the occurrence of *argenteus* samples in *graellsii* was confirmed in additional chicks sampled from the same islands but in colony areas where no *argenteus* breed, and collected by an experienced ornithologist (J. M. Pons pers. comm.). We are thus confident that the ARG haplotype is actually present in typical *graellsii* specimens. The high haplotypic diversity of *argentatus* is apparent from both samples of the taxon: northern Norway and Finland. All haplotypes found in the Norwegian sample were also observed in the Finnish samples. The Finnish birds were caught at refuse tips: they are not breeding birds, but only adults or near-adults were sampled. They were sampled by very experienced people who specialized in large gulls identification. No bird showed ambiguous phenotypes and identification problems can be excluded. The *mongolicus* specimens were adults caught on the breeding sites. No other large gull taxon breed in the same area. The *heuglini* and *vegae* specimens from the Stockholm museum where examined by L. Jonsson (pers. comm.) who did not detect any morphological sign of introgression with *L. hyperboreus*. The HYP haplotypes found in the samples of those taxa do not result from identification problems or inclusion of hybrids. The FUS haplotypes found in *vegae* and *heuglini* originate from specimens collected in Siberia in summer (on the breeding grounds of those taxa) and outside the range of *fuscus* or *graellsii*. Here again, hybrids or misidentified birds can be excluded. The only contentious case concerns the *heuglini* specimens from Finland (see above), but the fact that it had a haplotype that is rare in *fuscus* (one out of 18 birds) supports the proposed identification. Pictures and measurements of that bird are available for future reference.

Evolutionary history of the large white headed gulls.—The Artic species *L. hyperboreus*, *L. thayeri*, and *L. glaucoides* form together with *L. schistisagus* a well-supported clade in our phylogenetic analyses. Due to small sample sizes, it is difficult to exclude that horizontal gene transfer (through hybridization) explains those apparent relationships. A close relationship between *L. glaucoides* and *L. thayeri* is favored by most recent authors (e.g. see Sibley and Monroe 1990, American Ornithologists' Union [AOU] 1998) and *L. thayeri* has a juvenile plumage that can be very similar to *L. glaucoides kumlieni*, although its adult plumage is closer to *L. argentatus*. *Larus hyperboreus* is at all ages very similar to *L. glaucoides*. The clade formed by those three Arctic species in the mitochondrial tree thus probably records their recent common origin. On the contrary, *L. schistisagus* does not fit into that clade morphologically. Although the haplotypes of our two specimens are unambiguously included in the Arctic group, the fact that one of those haplotypes is identical to our *kumlieni* haplotype gives some support to the hypothesis of hybridization. Further discussion of the relationships within the Arctic species clade and of the position of *L. schistisagus* would require more specimens.

The *argentatus–fuscus* complex as a whole has been treated as a superspecies by Helbig (1997). Sibley and Monroe (1990) only placed *L. argentatus*, *L. cachinnans*, and *L. armenicus* into *Larus* (superspecies *argentatus*). Such taxonomic treatments imply that the taxa grouped in those superspecies are each other's closest relatives. On the contrary, our data suggest that the *argentatus–fuscus* complex as traditionally defined is not a natural group. First, the North American taxon *smithsonianus* seems to belong to a predominantly North American clade including also *L. californicus*. It might seem odd to link *smithsonianus* (and *L. californicus*) with the phenotypically strikingly different *L. hyperboreus* and *L. glaucoides* rather than with *L. argentatus*, which is indistinguishable in adult plumage. Nevertheless, *thayeri* and *kumlieni* have intermediate plumage characters between the white-winged, pale *glaucoides* and *hyperboreus* and the darker *smithsonianus*, in immature plumage as well as in adult plumage. Further-

more, previous results (Crochet et al. 2000) have shown the unreliability of adult plumage characters to determine phylogenetic relationships in gulls. Second, our results identify the Eurasian *L. marinus* as the sister taxon of *michahellis*. The low level of genetic variation between *L. marinus*, *L. argentatus*, *L. fuscus*, and *L. michahellis*, other than showing that they are closely related, prevents the determination of the relationships among those taxa. It is nevertheless obvious from the molecular data that a group including the latter taxa but not *L. marinus* would not be a monophyletic clade.

Even if that is not supported by the results presented here (Fig. 1), we cannot exclude that *marinus* and the Eurasian members of the *argentatus–fuscus* complex form a monophyletic group relative to the North American–Arctic clade. A common origin of *L. marinus*, *L. fuscus*, *L. argentatus*, and *L. michahellis* is even favored by some maximum-likelihood phylogenetic analyses of the molecular data (see fig. 1 in Crochet and Desmarais 2000). Furthermore, those species and *L. marinus* share very similar plumage at all ages, including the juvenal and immature plumages which are likely to represent species relationships better than adult plumages (Crochet et al. 2000).

Excluding the control region sequences which does not evolve in a clock-like manner in large gulls (Crochet and Desmarais 2000), the cytochrome-*b* haplotypes of the *argentatus–fuscus* complex differ by 0.34 to 1.99% sequence divergence. Using the usual calibration of 2% sequence divergence per million years for the cytochrome-*b* gene (Shields and Helm-Bychowski 1988) as a crude approximation, those haplotypes would have diverged between 1,000,000 and 170,000 years ago. Note that the species are likely to be even younger than that (e.g. see Edwards 1997). These amounts of genetic divergence are at the lower end of the range of avian interspecific distances (Helbig et al. 1995, Klicka and Zink 1997). They suggest that the species of the *fuscus* clade evolved comparatively recently, a fact that would account for the many controversial species limits in this group.

The large white-headed gulls of the *argentatus–fuscus* complex constitute one of the best known examples of ring speciation. According to this scenario (Mayr 1942, 1963), *L. fuscus* would have gradually differentiated as it colonized eastward across Eurasia, giving birth to the gradually paler forms that breed in Siberia. From there, North America would have been colonized by the ancestors of *smithsonianus*. The ring speciation would have been completed by migration from North America to Europe, giving birth to *argenteus* and *argentatus*. One crucial hypothesis of the circular overlap model of speciation in the LWHG is that the North American *smithsonianus* and the European *L. argentatus* should be sister taxa, and that *argenteus* and *fuscus* should be more distantly related. If confirmed with larger samples, the distant relationships between the North American *smithsonianus* and the European *L. argentatus* would clearly refute the suggestion that *argentatus* recently evolved from a transatlantic migration of *smithsonianus* after the separation of *L. fuscus* and *L. argentatus* and thus invalidate the ring species model for LWHG species.

Introgression of mitochondrial DNA.—Most haplotypes are found in more than one species of gull (Fig. 2, Table 4), indicating that either lineage sorting is still incomplete or mitochondrial gene flow occurs between the species. In the latter case, the observed polymorphism would have been retained since its origin, that is since the divergence of the lineages that can be found today a given species. It can be shown (Appendix; see also Tajima 1983, Avise 1994) that, for sample sizes between 10 and 50, the coalescence of the sampled haplotypes occurs with a probability of 0.95 within 4 Nf generations, where Nf is the effective female population size. In large gulls, that is about $4 \times 9 = 36$ Nf years using demographic data from *michahellis* (see Defos du Rau 1995). Based on the estimate of haplotype divergence time proposed above, it would thus take an effective female population size of more than 27,000 to retain some ancestral polymorphism between the most divergent lineages, but only slightly more than 4,500 between the most closely related lineages. Most species currently have census size above 100,000 pairs, but all have considerably increased in number in the last few tens of years ($<$10 gull generations; Hagemeijer and Blair 1997). Whereas some species might have maintained an effective population size $>$4,000 pairs, a long-term effective population size of 27,000 pairs is unrealistic. In conclu-

sion, although we cannot exclude some retention of ancestral polymorphism between the most closely related lineages, sharing of the most divergent lineages clearly result from introgression.

Lineage sharing between *L. hyperboreus* and the *argentatus–fuscus* complex can thus be safely attributed to gene flow after the origin of the Arctic species. Hybridization between *L. hyperboreus* and various taxa of the *argentatus–fuscus* complex has been reported in the wild, including in Arctic Russia, where we detected some instances of gene flow. (e.g. Ingolfsson 1970, Andrle 1980, Pierotti 1987). Snell (1991b) questioned the validity of the alleged cases of hybridization, suggesting that intrapopulation variability instead of hybridization might be responsible for the intermediate appearance of some specimens (see also reply by Ingolfsson 1993). Our data confirm that hybridization between *L. hyperboreus* and taxa of the *argentatus–fuscus* complex has led to exchange of mitochondrial genetic material.

The extent of lineage sharing between species that we detected in the present study is similar to the observations of Liebers et al. (2001) for other taxa of the LWHG complex. Those authors also suggest introgression as the explanation for their findings, especially because most instances of lineage sharing are between geographically adjacent taxa. In Liebers' study, as in the present study, some introgression has been detected between populations that are now fully allopatric (*graellsii* in ''*atlantis*–north'' [actually not *atlantis*] in Liebers' study, FUS et MAR in *michahellis*, MIC in *argentatus* and *vegae*, HYP in *mongolicus* in the present study). These probably originate from ancient contacts between those taxa in the past, especially during the glacial maxima where northern bird taxa has a more southern distribution (e.g. Blondel 1986, Alcover et al. 1992, Covas and Blondel 1998).

Efficiency of reproductive isolation in Western Europe.—The three sympatric European species *L. marinus*, *L. argentatus*, and *L. fuscus* have highly different haplotypes frequencies (see Tables 4 and 5 and Fig. 2), indicating that present mitochondrial gene flow is at most very limited. In mixed colonies in western Europe, haplotype segregation between *L. fuscus* and *L. argentatus* is nearly complete.

If gene flow is the only reason for lack of complete lineage sorting, crude estimates of the amount of mitochondrial gene flow can be approximated from the pairwise F_{ST} values between *L. fuscus*, *L. marinus*, and *L. argentatus*, using the equilibrium relationship between $F_{ST}$and Nm under the infinite-islands model hypotheses: $F_{ST} = 1/(1 + 2N_fm[n/n–1])$, with $n = 2$ the number of populations (see Rousset 1996 and Friesen et al. 1996). That amount is at most 0.5 migrant per generation between *L. marinus* and *L. argentatus* and 0.2 between *L. argentatus* and *L. fuscus*.

In the case of *L. hyperboreus* and *L. argentatus*, where gene flow is by far the most likely explanation for lineage sharing, we obtain a value of 0.2 females per generation. That would mean that at most one hybrid female successfully breeds every four generations, or every 36 years. Note that the frequency of hybridization might be much higher if most hybrid females are sterile, which could happen becuse they are the heterogametic sex in birds.

Systematics of the large gulls.—Using as a yardstick the amount of genetic differentiation between undisputed species (see above), we can attempt to derive some systematic conclusions from our data for the various form of uncertain status in the large gulls group. We follow the biological species concept and focus on the amount of gene flow and reproductive isolation. For fully allopatric taxa (e.g. North American and Eurasian taxa), phylogenetic relationships and past history are also taken into account to provide a consistent treatment of the taxa.

The form *michahellis* has long been treated as a subspecies of *L. argentatus* (e.g. Cramp and Simmons 1983). Following reports of its range extension on the western coast of France, leading to sympatric breeding with *L. argentatus* without significant hybridization (Yésou 1991), it has been separated as *L. cachinnans michahellis*, a status now widely accepted (Sibley and Monroe 1990, del Hoyo et al. 1996, Snow and Perrins 1997, AOU 1998). Observations from the contact zone between *michahellis* and *cachinnans* suggested that those two taxa should also be given specific status (Klein and Buchheim 1997). That has recently been confirmed by Liebers et al. (2001) who found a complete segregation of mitochondrial lineages between *L. michahellis* and *L. cachinnans* where they meet

in the western Black Sea area. Our genetic data confirm the distinctiveness of *L. michahellis* relative to the other western European species, especially *L. argentatus*. The main *michahellis* haplotype (MIC) is as divergent from the main *L. fuscus* (FUS) and *L. argentatus* (ARG) haplotypes as those last two are from each other. The level of differentiation and reproductive isolation of *michahellis* relative to *L. marinus*, *L. fuscus*, and *L. argentatus* is similar to the level of differentiation and reproductive isolation among those three undisputed species. The genetic distinctiveness of *michahellis* has also been claimed by Wink et al. (1994) on the basis of cytochrome-*b* gene divergence, but those authors had very little information on intraspecific polymorphism. However, we have no evidence that *michahellis* is more closely related to *L. fuscus* than to *L. argentatus*, as those authors proposed.

The presence of large gulls similar to Herring Gull but with yellow legs in the Gulf of Finland has been known to ornithologists since the early twentieth century (e.g. see Kumari 1978 or Jonsson 1998a for a review). Some authors considered that they represented a distinct taxon, distributed in the Gulf of Finland, the White Sea area, and the coasts of Murmansk: *L. argentatus omissus* Pleske, 1928 (e.g. Cramp and Simmons 1983, where it is included in the *cachinnans* group of subspecies), or *L. cachinnans omissus* (Haffer 1982). Others have suggested they were individuals of the form *cachinnans*, from the Black and Caspian sea areas, which colonized the eastern Baltic area during the twentieth century (see Kumari 1978). Whatever *omissus* might have been in the past, birds breeding at the present time in Finland and northern Norway are clearly *argentatus* (Mierauskas et al. 1991, 1994; Mierauskas and Greimas 1992; Jonsson 1998a). Our genetic data indicate that *L. argentatus argentatus* hybridized extensively in the east of its range with a taxon rather distantly related to the Herring Gull. The Finnish and eastern Norway populations of *argentatus* seem to result from an introgression between western Herring Gulls and a taxon carrying the 70 haplotype. That taxon—which is likely to be yellow-legged, because it would explain the introduction of ''yellow-legs'' genes into *L. argentatus*—could be *cachinnans* or *omissus*. Analyses of further samples should solve this matter.

Our results suggest that the North American form *smithsonianus* is more closely related to the North American–Arctic species *L. californicus*, *L. glaucoides*, *L. thayeri*, and *L. hyperboreus* than to *L. argentatus*. That taxon is generally treated as a subspecies of *L. argentatus*. On the basis of differences in immature plumage and in adult vocalization (according to Frings et al. 1958, who reported that European Herring Gulls do not react to the voice of their North American counterparts), Sangster et al. (1999) treated *smithsonianus* as a valid species. Our results agree with that hypothesis. The North American form *smithsonianus* is probably not the closest relative to the European *L. argentatus*, although adults of both species are extremely similar. Before recommending any taxonomic change, we would nevertheless prefer our results to be confirmed using larger sample sizes of all North American taxa to eliminate completely the possibility that the SMI haplotypes invaded *smithsonianus* from another taxon.

The specific status of *graellsii* is not supported by our genetic data. Populations of *fuscus* and *graellsii* share the same main haplotype, with no significant differences in haplotype frequency (see Table 5). There is no indication of restricted gene flow between *fuscus* and *graellsii*, and the lack of haplotype differences indicates that even if gene flow is currently restricted, separation of *fuscus* and *graellsii* is much more recent than of any valid species in the large gull group. We recommend the maintenance of *graellsii* as a subspecies of *fuscus*. We hypothesize that the marked morphological differences between these forms results from strong natural selection, acting against gene flow or after a complete but recent isolation. It should be emphasized that behavioral differences such as feeding ecology can arise very quickly and without genetic support, and that increasing awareness of the morphological variation in *fuscus* and *intermedius* questions the supposed clear-cut differences between those taxa.

The evaluation of the systematic status of the Siberian taxa that we investigated is complicated by extensive lineage sharing and a lack of original genetic material. All haplotypes detected in *vegae* and *mongolicus* are frequent in other species, one of them (HYP) clearly originating from introgression. In *heuglini*, we

found one haplotype (HEU) which is very rare in *L. fuscus* and absent in other species. Larger samples are thus required for any meaningful interpretation of the genetic data in these Siberian taxa.

Hybridization in birds.—Our data indicate that mitochondrial gene flow occurs between some of the large white-headed gulls but is generally low. Hybridization, however, can be more frequent than implied by the level of mitochondrial gene flow: if some postzygotic barriers exist, hybrids could have a lower survival, fertility, or both than their parents, reducing the genetic consequences of hybridization. Furthermore, according to Haldane's rule (Haldane 1922), hybrids of the heterogametic sex (females in birds) are expected to show reduced fitness compared to hybrids of the homogametic sex (males in birds). Nuclear gene flow (mediated by hybrids of both sexes) could thus be higher than mitochondrial gene flow (mediated by female hybrids only). That may explain the lack of differentiation observed for allozymic markers in gulls (Snell 1991a).

The lack of reciprocal monophyly observed between most of the large gull species is unusual for birds, but it is not unprecedented. Snow (*Anser caerulescens*) and Ross's (*A. rossi*) geese, which are known to produce fertile hybrids in nature (Cooke et al. 1995), were also found to share the same haplotypes, although in highly different frequencies, and secondary hybridization was also the favored explanation in that case (Avise et al. 1992). Extensive introgression of mitochondrial DNA has been documented in the Saker (*Falco cherrug*; Seibold et al. 1993), whereas the case of the Pomarine Jaeger (*Stercorarius pomarinus*) is still not settled (see Cohen et al. 1997 for a recent synthesis of phylogeny of skuas). In addition, several examples of limited introgression in areas of overlap have been published (*Uria* murres, Friesen et al. 1993; *Anas* ducks, Rhymer et al. 1994; *Vermivora* warblers, Gill 1997; *Pycnonotus* bulbuls, Lloyd et al. 1997).

Acknowledgments

We first would like to thank the many amateur or professional ornithologists who collected and sent samples to us. The French specimens collected by P.A.C. were obtained during culling performed by the Tour du Valat Biological Station, and organized by Nicolas Sadoul. The names of all contributors can be found in Table 2. B. Godelle provided us with the calculus used in the Appendix. E. Desmarais provided technical assistance. We thank P. Alström, S. Griffith, L. Jonsson, J. Merilä, B. Sheldon, F. Rousset, and A. Guillaumet for help with the manuscript.

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Associate Editor: F. Sheldon

APPENDIX. We determined the time needed to achieve the coalescence of n genes sampled in a population of N individuals.

For haploid genomes, the distribution of the probability that coalescence from n to $(n - 1)$ genes occurs in t generations can be approximated as an exponential distribution of parameter $n(n - 1)/2N$ (Li 1997). The coalescence time of the n genes is the sum of the time needed for each coalescence event (from n to $n - 1$, from $n - 1$ to $n - 2$, . . .).

The distribution of the coalescence time of the n genes is thus the distribution of a sum of $(n - 1)$ random variables, each one following an exponential distribution of parameter $n(n - 1)/2N$, $(n - 1)(n - 2)/2N$, . . . , $2/2N$.

It can be shown that the probability density function of a sum of random variables X_i each following an exponential distribution of parameter λ_i is $\Sigma_i\, 1 - e^{-\lambda_i t}/\Pi_{j \neq i}(1 - \lambda_i/\lambda_j)$.

Using this formula, we were able to plot the distribution of the coalescence time of the n genes (Fig. A1). As can be seen on the figure, for sample sizes routinely used in population studies (between 10 and 50 individuals), the time needed to achieve coalescence with a probability >0.95 and thus loose ancestral polymorphism is very close to $4N$ generations (between 3.9 and 4.1 × N generations).

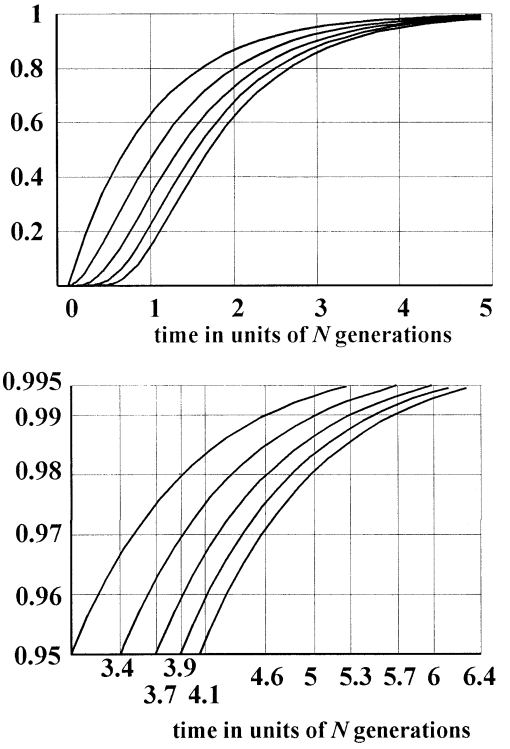

FIG. A1. Probability of coalescence of n sampled genes for n values of 2, 3, 5, 10, and 50 (from left to right). The lower curve focuses on the probabilities above 0.95. See the Appendix for explanations.