Genetic Affinities Within the Herring Gull *Larus argentatus* Assemblage Revealed by AFLP Genotyping

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Abstract: To date, the taxonomic status of circumpolar breeding populations of the Herring Gull *Larus argentatus,* the Lesser Black-backed Gull *Larus fuscus,* and the closely related Yellow-legged Gull *Larus cachinnans* has been based on differences or similarities in phenotype, morphology, and feeding and premating behavior. To shed some new light on the many taxonomic uncertainties surrounding these taxa, we describe the results of a large DNA study based on comparing the distribution of 209 biallelic markers among 109 gulls, representing 11 gull taxa of the Herring Gull assemblage and the Common Gull *Larus canus.* A detailed phylogenetic analysis failed to show clustering of individuals into groups representing either geographic origin or phenotype. Alternatively, birds were grouped into taxa defined on the basis of phenotype and geographic origin or phenotype alone. Genetic analyses revealed significantly different genetic distances between all pairs of taxa. However, based on these genetic distances, again no consistent phylogenetic tree could be constructed. Analysis of molecular variance indicated that about 77% of the total genetic variability among these gulls could be explained by within-taxon differences. Only 23% of the total genetic variability was due to genetic differences between taxa, irrespective of their species or subspecies status. Although this seems to challenge the current taxonomic treatment of the herring gull assemblage, our results are too premature and too incomplete to recommend a drastic change.

Key words: Phylogeny — *Larus* — Herring Gull assemblage — AFLP — Populations — Analysis of molecular variance (AMOVA)

Introduction

Understanding the taxonomic status of the various circumpolar breeding species of the Herring Gull *Larus argentatus* assemblage is one of the most complex and challenging problems in avian taxonomy (Dwight 1925; Kist 1961; Mayr 1963, Barth 1968, 1975; Haffer 1982; Cramp and Simmons 1983). Within the western Palearctic this assemblage harbors at least 14 gull taxa (the term "taxon" is used here to represent both currently recognized species and subspecies), including the Lesser Black-backed Gull *Larus fuscus* and the closely related, more southerly breeding Yellow-legged Gull *Larus cachinnans.* These taxa are recognized on the basis of differences in behavior, phenotype, and morphology (Haffer 1982). Clearly at odds with this apparent phenotypic differentiation are the results of two genetic studies, using a limited number of polymorphic allozyme loci (Snell 1991; Johnson 1985). Both studies revealed very little genetic differentiation between taxa, purportedly due to a recent common ancestry and/or high levels of gene flow. Several authors postulated that all extant taxa
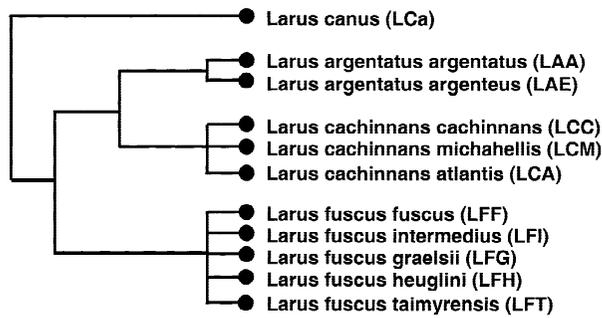
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Fig. 1. Phylogeny of the Herring Gull assemblage as proposed by Haffer (1982). This phylogeny is based on differences in behavior, biometry, molting strategy, migration patterns, and plumage.

of this Herring Gull assemblage are the result of a complex and rapid speciation process, which began at the onset of the last Ice Age and was finished shortly thereafter, approximately 10,000 years ago (Barth 1968, 1975; Voipio 1993). Mayr (1963), presented the geographical distribution of these gull taxa as a classical example of a ring-species complex where the two sympatric species (*L. fuscus* and *L. argentatus*) are the terminal links of the circumpolar chain. He postulated that all current taxa derive from four Pleistocene refugia. At present, most taxa seem reproductively isolated. Only during colonization of new areas does hybridization occur frequently, sometimes even leading to fertile offspring of the heterogametic sex—i.e., females in birds (van Swelm, unpublished results). In one well-studied case hybridization between *L. fuscus* and *L. argentatus* stopped as soon as *L. fuscus* became settled (Tinbergen 1953). However, hybridization between *L. cachinnans* with both *L. fuscus* and *L. argentatus* is still frequently observed 15 years after the initial settling of *L. cachinnans* in The Netherlands (van Swelm 1998, 2000; *contra* Yésou 1991).

We anticipated that if the phenotypic and behavioral differences between these taxa were indeed the result of a process of rapid reproductive isolation, slowly evolving lineage markers such as the commonly used mitochondrial cytochrome *b* gene would not display significant genetic differentiation (cf. Crochet et al. 2000; *contra* Wink et al. 1994). Recently, it has been shown that an AFLP-locus based phylogeny corresponded closely with differences in phenotypes among Lake Malawi cichlid fish (Albertson et al. 1999). For this reason, and to reduce the influence of genetic drift, differential introgression, and skewed sex-dependent colonization effects (for which all mtDNA-based polymorphisms are very sensitive), we decided to analyze the genetic contribution of both sexes by means of the recently developed AFLP method (Vos et al. 1995). Genotypes of 209 autosomal codominant inherited biallelic AFLP loci were obtained in 106 birds belonging to 11 Herring Gull taxa and three Common Gulls *Larus canus*. As such, this study describes the first large-scale DNA-based analysis specifically focusing on this notoriously complex—but at the same time fascinating—group of avian taxa.

Methods

Sample Collection

We largely follow the nomenclature of gull taxa as proposed by Haffer (1982) (Fig. 1). Over a period of 10 years, blood samples were collected at 14 sites, all close to or at a breeding colony (Table 1) by local experts to avoid identification errors. Samples were collected in 70% ethanol or EDTA and transferred to the Leiden University Medical Center as soon as possible. DNA was isolated by means of standard phenol/chloroform extraction. We obtained DNA of sufficient quality for the purpose of this study from 109 birds: 106 samples from 11 gull taxa of the Herring Gull assemblage and 3 samples of a smaller gull species, the Common Gull *Larus canus,* which we included as an outgroup in a number of analyses.

AFLP Analysis

The analysis of amplified fragment length polymorphisms (AFLP) allows the detection of RFLPs in genomes with unknown sequence structures (Vos et al. 1995). Essentially, AFLP is based on (i) restriction enzyme digestion of genomic DNA, (ii) ligation of the digested fragments with specific adapters and selective amplification by means of PCR, and (iii) gel analysis of the amplified fragments. For the present study AFLP reactions were carried out using the enzyme combination *Eco*RI and *Taq*I. Standard AFLP adapters for these restriction sites were ligated to the DNA fragments and AFLP primers for both enzymes were used in subsequent amplification reactions. The detection and subsequent analysis of the fragments closely followed the procedures outlined in detail previously (Vos et al. 1995). Following these protocols we obtained genotype scores of 209 distinct loci.

Statistical and Phylogenetic Analysis

After AFLP analysis two genetic scores were constructed. The first represents the genotype of each locus. Thus for each individual, 209 genotype scores were obtained—0/0, 0/1, and 1/1—which, in analogy with conventional RFLP scores, represent the absence (0) or presence (1) of a particular band. Since AFLP alleles, like RFLP alleles, are codominant inherited, one can distinguish two homozygous and a single heterozygous genotype. Based on these genotypes we also derived a haplotype score, 0–1–?, where 0 indicates the total absence of an AFLP fragment (genotype 0/0), 1 represents the presence (heterozygous or homozygous) of an AFLP fragment (genotypes 0/1 and 1/1), and ? represents unresolved scores (genotypes 0/?, 1/?, and ?/?). This haplotype score underestimates the total amount of genetic information present in our data set, thereby providing a conservative estimate.

First, all haplotype scores were analyzed by means of the PUZZLE v4.0 package (Strimmer and von Haeseler 1996, 1997). A single complete maximum likelihood-based tree resolving analysis with 100 intermediate trees on this total data set was performed. [This analysis took 96 h using a PC with a Pentium II MMX (233-MHz) processor; increasing the number of intermediate trees to 1000 would require an unrealistic >5000 h.] The resulting tree was drawn using TreeView v1.5.2 (Page 1998) and is illustrated in Fig. 2.

Second, we employed an analysis of molecular variance (AMOVA) by means of ARLEQUIN v1.1 (Excoffier et al. 1992; Schneider et al. 1997). This allows the partitioning of the total amount of genetic variation present in any data set into a number of hierarchical ordered components representing (1) genetic variance due to differences between individuals within populations, (2) genetic variance due to differences between populations within groups, and (3) genetic variation due to differences between groups. From a taxonomic perspective, this could be translated to differences between individuals of the same

Table 1. Information on the sampling locations and the taxa included in this gull DNA study

	Sampling location information			Taxon information	
No	Name (country)	Latitude	Longitude	Name (code)	Number of samples
1	Taimyr (Russia)	+74.07	+86.29	Taimyr Gull, *Larus fuscus taimyrensis* (LFT)	6
2	Kanin (Russia)	+67.80	+44.14	Heuglins Gull, *Larus fuscus heuglini* (LFH)	10
3	Kandalaksha (Russia)	+67.00	+32.30	Herring Gull, *Larus argentatus argentatus* (LAA)	5
4	East Finland	+61.50	+28.50	Baltic Gull, *Larus fuscus fuscus* (LFF)	3
5a	West Finland	+60.07	+25.25	Herring Gull, *Larus argentatus argentatus* (LAA)	7
5b	West Finland	+60.07	+25.25	Baltic Gull, *Larus fuscus fuscus* (LFF)	4
6	West Norway	+58.03	+6.40	Lesser Black-backed Gull, *Larus fuscus intermedius* (LFI)	10
7a	Maasvlakte (The Netherlands)	+51.51	+4.03	Common Gull, *Larus canus* (LCa)	3
7b	Maasvlakte (The Netherlands)	+51.51	+4.03	Herring Gull, *Larus argentatus argenteus* (LAE)	8
7c	Maasvlakte (The Netherlands)	+51.51	+4.03	Lesser Black-backed Gull, *Larus fuscus graellsii?* (LF?)[a]	10
8	West England	+53.30	−2.34	Lesser Black-backed Gull, *Larus fuscus graellsii* (LFG)	6
9	Faroe Islands	+62.14	−6.37	Lesser Black-backed Gull, *Larus fuscus graellsii* (LFG)	5
10	Iceland	+64.05	−21.59	Lesser Black-backed Gull, *Larus fuscus graellsii* (LFG)	9
11	Berlenga Isles (Portugal)	+39.25	−9.28	Atlantic Gull, *Larus cachinnans atlantis* (LCA)	5
12	Gibraltar (Spain/England)	+36.07	−5.17	Yellow-legged Gull, *Larus cachinnans michahellis* (LCM)	6
13	Essaouira (Morocco)	+31.32	−9.45	Atlantic Gull, *Larus cachinnans atlantis* (LCA)	5
14	Ukraine	+46.24	+32.03	Steppe Gull, *Larus cachinnans cachinnans* (LCC)	7

[a] The Lesser Black-backed Gull race in The Netherlands could be LFG, but LFI gene flow cannot be excluded based on plumage details: hence its uncertain status.

subspecies, between subspecies within species, and between species. As such, this allows the testing of any a priori taxonomical hypothesis looking for genetic differentiation between species and/or subspecies. Between genetically sufficiently isolated but related taxa, the majority of the genetic variance would be expected to be found between species, less so between subspecies, and little genetic variance between individuals of the same subspecies. The haplotype scores were grouped into a variable number of taxa on the basis of generally accepted phenotypic and/or geographic criteria as proposed by Haffer (1982) (Fig. 1, Table 1). By means of AMOVA we tested a number of hypotheses. The resulting decomposition of the variance components representing the various genetic variance estimates is summarized in Table 2. By means of AMOVA we also estimate an F_{st} analogue, ϕ_{st}, which not only considers differences in haplotype score frequencies, but also includes molecular differences between individual haplotype scores. On the basis of the pairwise ϕ_{st} distance matrix, an UPGMA tree was made using the UPGMA option contained in the NEIGHBOR routine included in PHYLIP v3.57c (Felsenstein 1995) and TreeView v1.5.2 (Page 1998) (Fig. 3A). Bootstrap supports were obtained by creating, from the original haplotype scores, 100 randomized sets of haplotype scores by means of the SEQBOOT routine included in PHYLIP v3.57c. Each of these 100 sets was again analyzed by means of ARLEQUIN v1.1, the resulting 100 pairwise ϕ_{st} distance matrices were combined into a single file, analyzed as above, and the resulting 100 trees were analyzed by means of the CONSENSE routine, also included in PHYLIP v3.57c. Finally, again using ARLEQUIN v1.1, we estimate the number of pairwise differences (often referred to as the mismatch distribution) between all originally observed haplotype scores (Fig. 4).

Since the haplotype scores represent an underestimate of the true genetic variability in our data set, we also analyzed the genotypic scores. Using GDA v1.0 (d12) (Lewis and Zaykin 1999), GENEPOP v3.1c (Raymond and Rousset 1995), and ARLEQUIN v1.1, a number of descriptive statistics were obtained for all loci in all taxa (Table 3). These include, for each taxon, H_e, the expected heterozygosity, H_o, the observed heterozygosity, N_m, the estimated number of migrants per generation between taxa, F_{is}, Wright's inbreeding coefficient, and the allele frequency distributions. In addition, we calculated, across all loci and all taxa again, F_{is} (inbreeding within taxa), F_{it} (overall inbreeding), and the coancestry coefficient F_{st}, which provides an estimate of interspecific genetic differentiation. From the allele frequencies of all 209 loci, we calculated Nei's (1972) genetic distance by means of the

GENDIST routine from PHYLIP v3.57c. The resulting distance matrix was analyzed exactly as the haplotype score-based ϕ_{st} matrix with one difference: the bootstrap supports were obtained on the basis of 1000 SEQBOOT-generated sets of allele frequencies. The resulting tree is illustrated in Fig. 3B.

Results

A maximum likelihood-based phylogenetic tree connecting all individuals on the basis of the individual haplotype scores was constructed by means of PUZZLE v4.0 (Fig. 2). No consistent grouping of individuals into distinct clusters (e.g., representing the sampling locations or phenotype) was observed. In contrast, most individuals form a distinct branch of their own, and all branches seem to descend from a single shared ancestor in a star-like fashion. This strongly suggests a relatively recently shared common ancestry between all herring gulls and/or a rapid expansion. This is confirmed by the mismatch distribution (Fig. 4). The unimodal shape of this number of pairwise difference distribution is a good indication of a single-exponential population expansion.

We subsequently grouped all birds into a number of taxa on the basis of their phenotype and/or geographical origin. On the basis of the haplotype scores, the genetic distance (ϕ_{st}) between all pairs of taxa was estimated by means of AMOVA (Table 2). When birds were grouped into a single group containing 17 gull taxa identified by the combination of sampling location and phenotype, about 78.4% of the total genetic variability present among all birds could be attributed to genetic differentiation *within* taxa, whereas the remaining 21.6% was due to genetic differences *between* taxa. A similar partitioning was obtained when birds were allocated to 12

Fig. 2. A quartet puzzling based maximum-likelihood tree of all 109 gulls based on the binary AFLP haplotype scores. Branch supports (most of which are below 50%) are removed for clarity.

taxa solely on the basis of their phenotype. We also analyzed a number of more detailed models representing several previously proposed taxonomic orderings. In all these alternative models the within-taxon genetic variance explained about 77% of the total genetic variance, and the remaining 23% was due to the between-taxa, within-groups and the between-groups components.

Again, this indicates that among our birds, despite their geographical and phenotypical differentiation, no specific genetically well-defined taxa could be identified. Thus, on the basis of our haplotype score-derived AMOVA analysis, no clear hierarchical difference in the between-species and between-subspecies contributions could be detected, although when the two most extreme models were compared—model 3 with four Herring Gull species and model 6 with seven species—the latter model did indicate a slightly better species–subspecies structure.

Table 2. Results of analysis of molecular variance (AMOVA) based on haplotype scores and genotypes (in parentheses) of 209 AFLP loci among all 109 gulls

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 6
				Number of groups defined			
	1	1	4	4	5	7	8
Populations in groups	Taxa (n = 17) defined on the basis of phenotype and sampling locations (Table 1) included in a single group	Taxa (n = 12) defined on the basis of phenotype only (Table 1 and Figs. 1 and 2) included in a single group	G1: LCa G2: LAA, LAE G3: LCC, LCA, LCM G4: LFG, LF?, LFI, LFF, LFH, LFT	G1: LCa G2: LCC G3: LCA, LCM, LAA, LAE G4: LFG, LF?, LFI, LFF, LFH, LFT	G1: LCa G2: LAA, LAE G3: LCC G4: LCA, LCM G5: LFG, LF?, LFI, LFF, LFH, LFT	G1: LCa G2: LAA, LAE G3: LCC G4: LCA, LCM G5: LFG, LF? G6: LFI, LFF G7: LFH, LFT	G1: LCa G2: LAA, LAE G3: LCC G4: LCA, LCM G5: LFG, LF? G6: LFI G7: LFF G8: LFH, LFT
			AMOVA results expressed as % genetic variance due to differences				
Between groups	— (—)	— (—)	11.9 (10.5)	12.9 (11.7)	13.4 (12.1)	14.3 (12.3)	15.8 (13.6)
Between populations, within groups	21.6 (22.5)	20.0 (19.9)	11.4 (12.3)	11.1 (11.8)	10.1 (10.9)	6.7 (8.5)	5.2 (7.1)
Within populations	78.4 (77.5)	80.0 (80.1)	76.7 (77.2)	76.0 (76.5)	76.5 (77.0)	79.0 (79.2)	79.0 (79.3)

It could be argued that the AFLP haplotype score, because of its recessive character, underestimates the genetic information content. Therefore, we repeated all analyses using the AFLP genotypes. Table 3 summarizes a number of descriptive statistics. Very striking is the difference between H_e and H_o. For all taxa H_o was less than 50% of H_e, indicating a marked reduction in the number of heterozygotes. This and the high F_{is} (inbreeding coefficient) values are good indications of a high proportion of birds returning to the same breeding colony. On the other hand, the rather high N_m values and the very few private alleles (N_{pa}) observed (13 in total) indicate high levels of gene flow. This is also confirmed by the three coefficients, F_{is} (0.61), F_{it} (0.67), and F_{st} (0.16) (not shown), which are all highly significant indicators of little geographic and interspecific differentiation. These results are confirmed by our hierarchical AMOVA (Table 2), which shows near-identical results when the genotype-based data are compared with the haplotype score analyses.

Two UPGMA trees were built on the basis of the AMOVA-derived pairwise ϕ_{st} distances. One represents the genetic distances estimated from the haplotype scores. The other represents Nei's (1972) genetic distance, based on the genotypes (Figs. 3A and B). Both trees have a structure closely corresponding with the species/subspecies hierarchy proposed by Haffer (1982) (Fig. 1). All Lesser Black-backed Gull taxa (*L. f. fuscus, L. f. intermedius, L. f. graellsii, L. f. heuglini,* and *L. f. taimyrensis*) form a single major clade, whereas the two

Herring Gulls (*L. a. argenteus* and *L. a. argentatus*) and two Yellow-legged Gull taxa (*L. c. michahellis* and *L. c. atlantis*) form subclades in a second major clade. The Steppe Gull *L. c. cachinnans* is paraphyletic to all other large gulls and closest to the outgroup (Common Gull *L. canus*). Only three branches, those connecting (1) *L. f. graellsii* from Iceland, the Faeroe Islands, and England with Dutch *L. f. graellsii,* (2) *L. a. argenteus* and *L. a. argentatus,* and (3) *L. c. michahellis* and *L. c. atlantis,* have support values >90%. Especially the support for the bifurcation of the two major groups is rather low in both trees (42 and 30%). This suggests that these taxa are extremely closely related to one another and that a complete and reliable phylogenetic ordering of these gulls on the basis of the present genetic information is impossible.

Discussion

In this study we describe a detailed genetic analysis of western Palearctic taxa of the Herring Gull *Larus argentatus,* the Lesser Black-backed Gull *Larus fuscus,* and the Yellow-legged Gull *Larus cachinnans.* These birds, representing the notoriously complex Herring Gull assemblage, have been the subject of heated debates throughout the past century. Taxa have been shifted between almost all possible taxonomic statues off and on (Dwight 1925; Kist 1961; Barth 1968, 1975; Haffer 1982; Cramp and Simmons 1983). Invariably, new in-
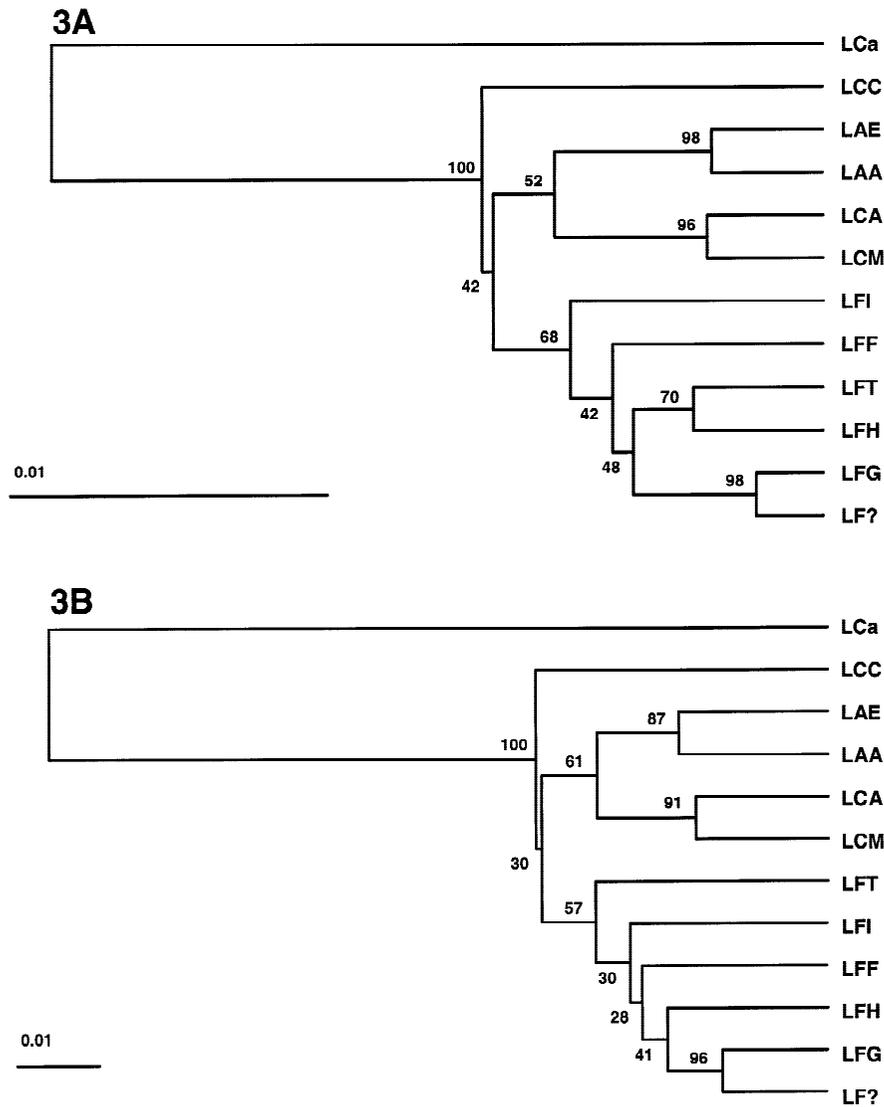
3A

0.01

3B

0.01

Fig. 3. **A** UPGMA tree based on the AMOVA-derived ϕ_{st} distance between pairs of gull populations based on the AFLP haplotype scores. Numbers of supporting bootstrap replications (as a percentage of 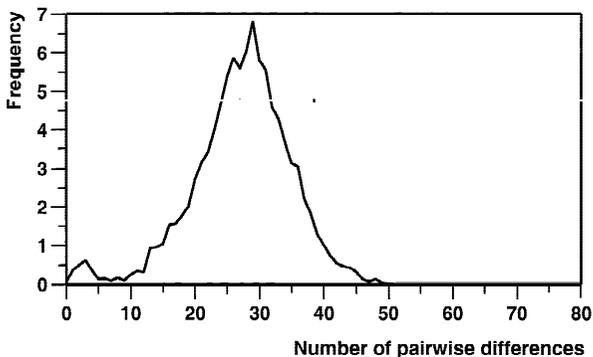100 replicates) are indicated to the *left* of bifurcations. **B** UPGMA tree based on Nei's genetic distance between pairs of gull populations based on the AFLP allele frequencies. Numbers of supporting bootstrap replications (as a percentage of 100 replicates) are indicated to the *left* of bifurcations.

Fig. 4. Mismatch distribution showing the number of pairwise differences between the 106 binary AFLP haplotype scores of the Herring Gull assemblage. The scores of the three Common Gulls were excluded from this analysis.

sights resulting from comparative studies encompassing differences and/or similarities in phenotype and various behavioral aspects immediately resulted in a new taxonomic rearrangement. Taxa are even being declared spe-

cies or completely removed from the taxonomic landscape solely on the basis of personal preference (Sangster et al. 1999). Therefore, it seemed wise to seek help by means of molecular methods. We speculated that if the phenotypic and behavioral differences between these taxa were indeed the result of reproductive isolation, this could be reflected by corresponding genetic differentiation.

We anticipated that an mtDNA-based analysis would not render sufficiently reliable information for a number of reasons. Because of the lack of any published autosomal genetic sequence information on large gulls, we used the recently published AFLP method allowing the analysis of many biallelic loci in genomes with an unknown sequence composition (Vos 1995). In addition, AFLPs have the advantage of representing genetic information from both sexes. Thereby, phenomena such as differential introgression, lineage sorting, and selective transmission due to skewed migration patterns, which

Table 3. Descriptive statistics based on genotypes of 209 AFLP loci among 109 gulls[a]

Population	N_{ind}	M_{ss}	P	H_e	H_o	N_m	F_{is}	N_{pa}
LCa	3	2.87	0.20	0.10	0.04	0.6	0.68	2
LAA	12	10.07	0.58	0.19	0.06	2.5	0.70	3
LAE	8	7.39	0.45	0.17	0.06	2.0	0.66	1
LCC	7	6.55	0.39	0.15	0.06	1.7	0.60	2
LCA	10	7.70	0.38	0.15	0.06	2.3	0.61	—
LCM	6	5.59	0.31	0.12	0.06	1.6	0.56	—
LF?	10	9.36	0.59	0.19	0.07	3.2	0.63	—
LFG	20	17.28	0.68	0.19	0.09	2.9	0.53	4
LFI	10	8.91	0.50	0.17	0.07	2.2	0.60	1
LFF	7	6.64	0.47	0.17	0.07	2.4	0.62	—
LFH	10	9.15	0.57	0.19	0.07	2.8	0.62	—
LFT	6	5.59	0.49	0.19	0.07	2.2	0.63	—
Average	9.1	8.10	0.47	0.17	0.07	2.2	0.62	

[a] N_{ind}, number of individuals analyzed; M_{ss}, mean sample size over all loci; P, proportion of polymorphic loci; H_e, expected heterozygosity; H_o, observed heterozygosity; N_m, average estimated number of migrants between taxon X and all other taxa; F_{is}, inbreeding coefficient within taxa; N_{pa}, number of private alleles for each taxon. Estimates were obtained by GDA v1.0 (d12) except N_m, which was obtained by ARLEQUIN v1.1.

can seriously distort true genetic relations between closely related taxa, can be avoided.

Our analyses indicate high levels of gene flow, with average N_m values varying between 0.6 and 3.2, but with values up to 8.3 for some population pairs. We also observed very few private alleles (only 13 of 418), and only 23% of the total genetic variance could be explained by genetic differentiation between taxa. These observations all indicate a minimal genetic divergence between closely related taxa. On the other hand, we observed very high inbreeding coefficients and significantly reduced heterozygosity values, both suggesting genetic isolation between taxa. These seemingly contradicting observations could be due to a combined effect of a recent common ancestry and a high breeding-site fidelity among these minimally divergent taxa. Our data confirm the results of two allozyme studies, which included partially the same taxa (Snell 1991; Johnson 1985). These authors also reported very low average heterozygosities and little interspecific differentiation. Only if we ignore the weak statistical support values does the topology of our UP-GMA trees closely match the phylogenetic ordering as proposed by Haffer (1982).

How can we reconcile the little genetic differentiation with the marked phenotypical differences and almost-complete reproductive isolation? Two models could explain this apparent discrepancy between phenotype and genotype in these gulls. The first model suggests that these birds have not yet completed an ongoing process of speciation. An early geographical isolation, before the last glacial period in Europe, was sufficiently long ago to result in the subtle phenotypical variability and near-complete reproductive isolation, but too recent to create a sufficient overall genetic differentiation between all the taxa when many recombining loci are analyzed. Also, the recent population expansions of some of these taxa could have forced many individual birds to cross the species boundaries, as reflected by the relative high N_m values (up to 8.3; not shown) between all taxon pairs except those involving the outgroup (and regularly observed in the field!).

The second model postulates that extant gull taxa are representatives of a single species with various forms sharing a recent common ancestry, explaining their close genetic affinities. The phenotypical variability of these forms could then be explained by various degrees of hybridization between a number of original founding populations. This must have occurred on different time scales and/or at different phases of environmental adaptation. The latter model was predicted by Barth (1968), based on phenotypic and morphological differences, and is not unlike the genetic history of the Noctule Bat (*Nyctalus noctula*) or our own human species. In both cases about 70–80% of the total genetic variability could be explained by genetic differences within geographically structured populations which spread throughout Europe shortly after the last Ice Age (Petit et al. 1999; Barbujani et al. 1997). At odds with Barth's hypothesis, proposing the Baltic Gull *L. f. fuscus* as the most likely ancestral taxon, is our finding suggesting that the Steppe Gull *L. c. cachinnans* is the oldest taxon of this assemblage. Although the bootstrap support for the paraphyletic status of this taxon does not exceed 50%, this seems to support the suggestion of Voipio (1993) that the Steppe Gull *L. c. cachinnans* is the oldest taxon in the Herring Gull assemblage. According to the ring-species concept (Mayr 1963), all extant Herring Gulls descend from four Pleistocene refugia, the oldest of which was found in the Aralo-Caspian area, where the Steppe Gull *L. c. cachinnans* still breeds. Although our AFLP data do not suggest a number of different founder populations, one could argue that the time span between the Pleistocene settling of three refugia out of the Aralo-Caspian founder population and the post-Glacial dispersal of modern Herring Gulls was too short to be detected by our loci. Hence, on the basis of our data, Mayr's hypothesis cannot be rejected.

Before deciding which model is favored, a more central question has to be answered: Can AFLP data and/or the AMOVA models be used to identify species limits? It has already been shown that AFLP loci can be used to obtain detailed insight into the evolutionary history of closely related species (Albertson et al. 1999), leaving little doubt about this first point. On the other hand, AMOVA has not yet been used routinely to explore phylogeographic and evolutionary hypothesis. We know of only one other avian AMOVA: the mtDNA-sequence study among races of the Dunlin (*Calidris alpina*) (Weenink et al. 1996). This study is even more interesting because it involves a similar number of closely re-

lated taxa, which have a geographical (circumpolar) breeding distribution and evolutionary history similar to those of our gull taxa. Among the Dunlin races, an mtDNA-based AMOVA revealed that about 70% of the total genetic variation could be attributed to differences between the taxa, and only about 30% was due to differences within taxa. Thus, AMOVA can reveal distinct genetic differentiation between closely related and recently derived avian taxa. Furthermore, it could be argued that, because the Dunlin AMOVA results were based on mtDNA-sequence information (which normally displays a markedly faster genetic divergence compared with autosomal loci), they are not in conflict with our results, simply because of the difference in the choice of loci. Hence, our finding of very little genetic differentiation between the gull taxa does not necessarily preclude acceptance of the first model but cannot reject the second one either.

On the basis of the currently available genetic data, we favor a hybrid between both models. Although the phylogeographic history of the Herring Gull assemblage, as hypothesized by Barth (1968), Mayr (1963), and Voipio (1993), is probably not entirely correct, it is probably not far off either. All three authors propose the Aralo-Caspian area as the most ancient region of origin of modern Herring Gulls. This corresponds with our results indicating that the Steppe Gull *L. c. cachinnans,* which still breeds in this area, could be the oldest taxon of this assemblage.

One could argue that all current sympatric-breeding, phenotypically distinct Herring Gull taxa are reproductively isolated and therefore, acknowledging the biological species concept, should be considered separate species. However, our AMOVAs did not reveal a distinct differentiation between the currently recognized species and subspecies. Because of this, a choice between the two extremes (three vs seven species) cannot be made. Therefore, we think it is wise to adopt the most conservative model, treating these taxa as three distinct species, *L. argentatus, L. cachinnans,* and *L. fuscus,* a conclusion which was proposed by Haffer in 1982 (see Fig. 1) and is still largely followed by most authors.

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