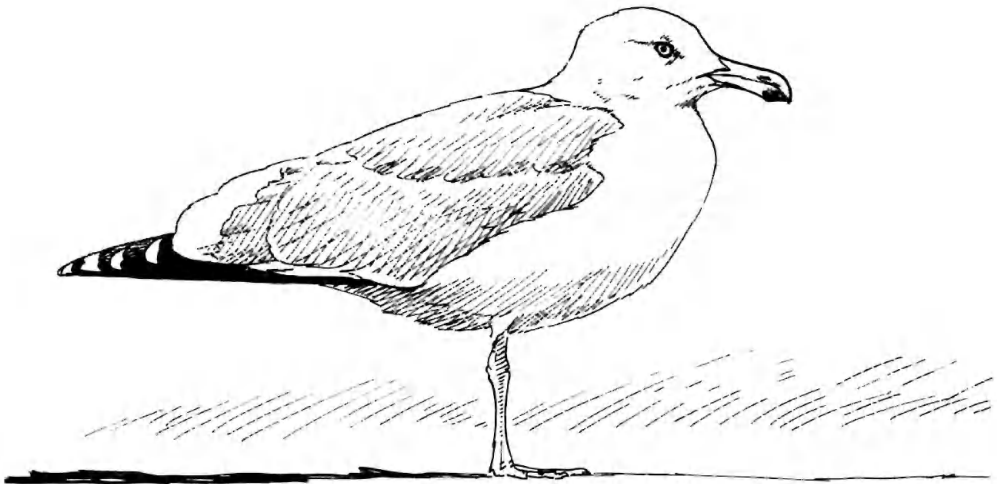




Genetic relationships among the different races of Herring Gull, Yellow-legged Gull and Lesser Black-backed Gull

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ABSTRACT Genetic analyses of the relationships among different forms of Lesser Black-backed Gull *Larus fuscus*, Herring Gull *L. argentatus* and Yellow-legged Gull *L. cachinnans* are beset with difficulties, associated with apparently low levels of genetic divergence, inbreeding, and the highly unstable nature of gull populations. This short review summarises and discusses a new attempt to tackle this problem (de Knijff *et al.* 2001). While the results do not solve the 'gull problem', they have provided much new evidence on the extent of genetic variation among different individuals of the same taxon, which will be crucial to any future consideration of gull taxonomy.

The complex of 'large white-headed gulls', which in Europe comprises the various forms of Lesser Black-backed Gull *Larus fuscus*, Herring Gull *L. argentatus* and Yellow-legged Gull *L. cachinnans*, has long been a source of lively taxonomic debate. There are at least 14 recognised taxa that potentially occur in the Western Palearctic, and probably at least as many interpretations of their specific status (e.g. Mayr 1963; Barth 1975; Cramp & Simmons 1983; Haffer 1982). A conservative viewpoint, such as that currently held by the British Ornithologists' Union, recognises only two species, Herring Gull and Lesser Black-backed Gull, each with a number of subspecies. In the climate of phylogenetic splitting which has accompanied the resurgence of interest in taxonomic matters among birdwatchers in Europe, the opposite (extreme) viewpoint has been to assume that all the recognisable forms are, in fact, separate species, and to treat them as such. A straw-poll among European birders would, however, probably reveal a consensus view that, in the Western Palearctic, four or five species exist:

- (1) Herring Gull *L. argentatus*, including the subspecies *argentatus*, *argenteus* and also, usually, *smithsonianus* ('American Herring Gull').
- (2) Lesser Black-backed Gull *L. fuscus*, including the subspecies *graellsii*, *intermedius* and *fuscus* (the latter sometimes being treated separately as 'Baltic Gull'). This group may also include the Siberian gulls *L. (f.) heuglini* and *L. (f.) taimyrensis*, since many adult *heuglini* are essentially indistinguishable from *graellsii* in the field.
- (3) 'Steppe Gull' or 'Caspian Gull' *L. cachinnans*, comprising the forms *cachinnans* (including the form '*ponticus*') and *barabensis*, although this category is often considered to include those taxa in (4) below.
- (4) 'Yellow-legged Gull' *L. (c.) michabellis*, including the forms *michabellis*, *armenicus* and *atlantis*.

In addition, some authors regard 'Siberian Gull', comprising the forms *heuglini* and *taimyrensis*, as potentially deserving of specific status, as *L. heuglini* (e.g. Filchagov *et*

al. 1992; Buzun in press).

It is, however, possible to argue about the true status of every taxon, and the relationships within this gull assemblage are best regarded as 'unproven'. Most authors agree that, during the Pleistocene glaciations, this group of large gulls must have been confined to three or four southern refugia, and that the northern forms have evolved as the birds moved out from these refugia during interglacial periods (Mayr 1963). Several research groups have undertaken genetic or biochemical studies on these gulls (Johnson 1985; Snell 1991; Wink *et al.* 1994; Heidrich *et al.* 1996; Liebers & Helbig 1999), with varying, but often equivocal, results which probably reflect either a high degree of gene flow among the various taxa or a very recent common ancestry, or both. Liebers & Helbig, using analyses of mitochondrial DNA (mtDNA), have produced the most promising and authoritative genetic results; since mtDNA is carried only through the female line (none of the spermatozoon's mitochondria enters the egg at fertilisation), however, there are potential problems with its use in situations where males and females behave differently or may have different levels of fertility in hybrid crosses.

A recent paper in *Journal of Molecular Evolution*, by de Knijff *et al.* (2001), re-examines the genetic relationship among 11 of these gull taxa (Common Gull *L. canus* was chosen as a more distantly related outgroup), using a modification of genetic fingerprinting known as Amplified Fragment Length Polymorphisms (AFLP). This method, which is explained more fully below (see Appendix on page 528), has the potential to circumvent some of the problems associated with studies by other workers. First, it looks at nuclear genes, hence reflecting the evolution of both sexes. Secondly, some of the sequences analysed will be evolving rapidly, thus allowing phylogenetic information to be obtained from taxa that have only recently diverged.

A criticism of some genetic studies, as described in Collinson (2001), is that the intra-taxon genetic variation is not examined properly, or is ignored altogether. It is not possible to assess the significance of genetic variation among individuals of different subspecies unless the average amount of varia-

tion among individuals in the *same* subspecies is known. To address this, de Knijff *et al.* took DNA from blood samples of 109 individual gulls from colonies in 14 Western Palearctic locations (fig. 1), subjected them to AFLP fingerprinting, and then analysed the amount of genetic variation not only between different taxa, but also within these taxa at the same site, and between geographically separated populations of the same taxa. As expected, different individuals had different genetic fingerprints, and these varied both within and between taxa, and between geographical locations. What was perhaps less expected was the degree of genetic variation *within* certain taxa, as opposed to the level of differentiation *between* taxa. The authors used statistics (the analysis of molecular variance, AMOVA, which is a modification of the analysis of variance test, ANOVA) to split the variation among different groups into quantified components. There are several different models by which the European large white-headed gulls may be grouped, and the authors tested the seven most sensible, described below.

Model 1 Treating every gull taxon from every sampling location (each point in fig. 1) independently: for example, not only are *cachinnans*, *michabellis*, *graellsii* etc. all analysed independently, but *graellsii* from different study sites (e.g. in England, Faroes and Iceland) are also treated independently in the analysis.

Model 2 Treating each taxon independently, but ignoring sampling location. Using the above example, *cachinnans*, *michabellis*, *graellsii* etc. would still be treated separately, but, in contrast to

Model 1, the *graellsii* from all locations would be combined into one group and analysed together.

Other models put all the gull taxa into groups that may represent putative species, and then look at genetic variation within and among the groups ('species') and among different populations ('subspecies') within the groups, as well as among different individuals of the same subspecies:

Model 3 Four groups of gulls representing four possible species. Common Gull is allocated a group of its own; *argentatus* and *argenteus* are lumped together; the 'yellow-legged gulls', i.e. *cachinnans*, *michabellis* and *atlantis*, are similarly grouped; while the 'black-backed gulls', including *beuglini* and *taimyrensis*, are placed in the fourth group.

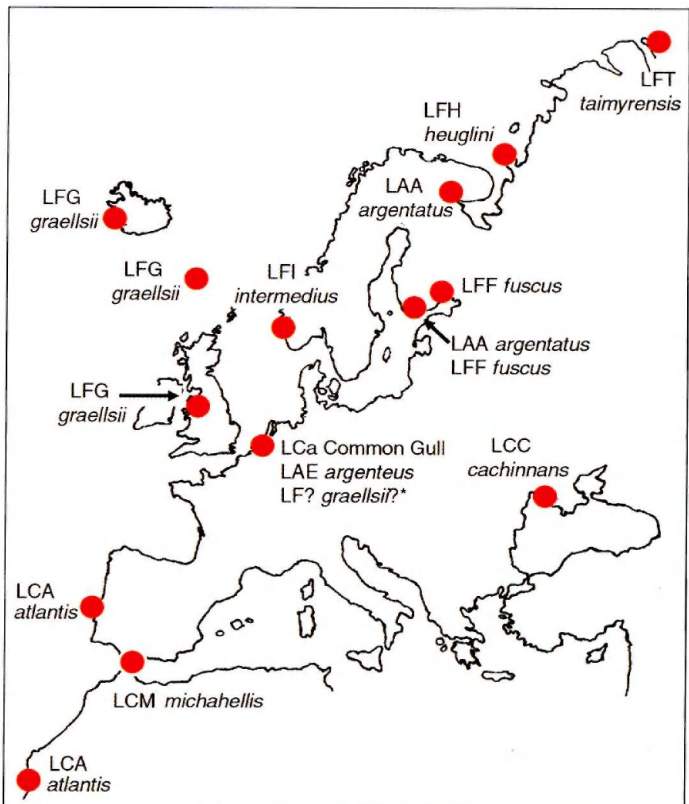


Fig. 1. Sampling locations of gulls *Larus* used in the study by de Knijff *et al.* (2001). The three-letter codes are shorthand for the full scientific names, so that 'LFG' represents *Larus fuscus graellsii*, although the subspecific names are repeated in full for clarity. Lesser Black-backed Gulls from the Dutch site (* in figure) are phenotypically similar to *graellsii*, but were designated 'LF?' to indicate that *intermedius* influence could not be eliminated, and were analysed separately.

Model 4 Four groups. Common Gull and *cachinnans* are placed in groups on their own; *michabellis* and *atlantis* are grouped with *argenteus* and *argentatus*; and, as in Model 3, all the 'black-backed gulls' are considered together.

Model 5 Five groups. As Model 3, but *cachinnans* is split from the other 'yellow-legged gulls', and placed in a category of its own.

Model 6 Seven groups. As Model 5, but with the 'black-backed' group split into three putative species: *graellsii* was lumped with the uncertain taxon (presumed *graellsii*) from The Netherlands; *fuscus* and *intermedius* were grouped together; and the 'Siberian' gulls, *heuglini* and *taimyrensis*, were treated as a separate group.

Model 7 Eight groups. As Model 6, but with *intermedius* and *fuscus* placed in different groups, on their own.

All the models produced very similar results. For each model, 76-80% of the genetic variance observed was due to differences *within* the populations studied (i.e. among individuals of the *same* taxon), with only the remaining 20-24% assignable to differences *among* groups (or among different subspecies within groups).

Therefore, although different forms of these gulls from different geographical locations are often easily distinguishable phenotypically, they do not form a genetically well-defined hierarchy of species, subspecies and individual variation. The results of the genetic analyses did not distinguish whether there are just two species (Herring Gull and Lesser Black-

backed Gull) or as many as seven.

When the authors drew a phylogenetic tree for all the different individuals sampled, this failed to produce any sensible groupings of similar-looking gulls from the same location into closely related clusters. Instead, the tree suggested a rather random divergence of all the gulls from a recent, single ancestor. The AMOVA analysis, however, allows the genetic differences among gull taxa to be isolated from the total genetic variation within populations, and enables phylogenetic trees to be drawn up on the basis of this inter-taxa variance. Such a tree, based on this analysis, is presented in fig. 2, and this looks more like what one might expect. The data suggest that, with Common Gulls as the outgroup, 'Caspian Gulls', *L. c. cachinnans*, are the sister group to all the other forms considered, with Herring Gulls of the subspecies *argenteus* and *argentatus* most closely related to western Yellow-legged Gulls *michabellis* and *atlantis*. The 'black-backs' form a clade of their own, with the Siberian

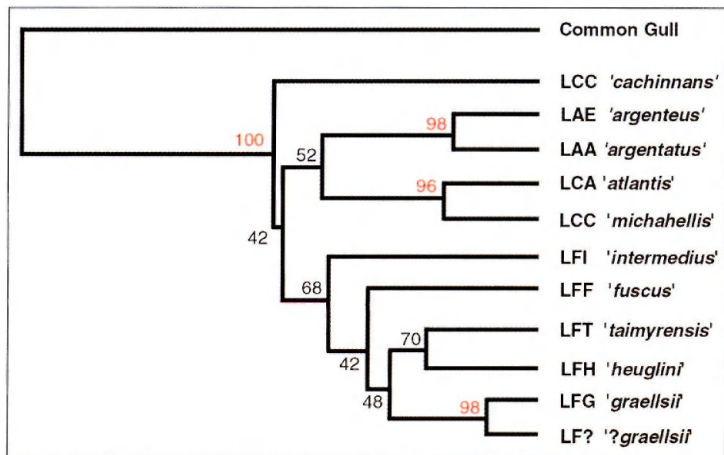


Fig. 2. Representation of the possible evolutionary history of the Western Palearctic large white-headed gulls *Larus* based on analysis of the AMOVA-derived genetic distance between gull populations. A measure of the degree of confidence (0-100%) that can be placed on the position of each bifurcation in the tree is given by the numbers, which represent the percentage of bootstrap replications that support the split. (Following de Knijff *et al.* 2001)

Although the tree looks convincing, most of the relationships outlined are not strongly supported. There is, however, strong support for the following groupings, marked in red:

- (1) *argenteus* and *argentatus*, the West European and Scandinavian Herring Gulls, are sister taxa.
- (2) *atlantis* and *michabellis*, the Atlantic and Mediterranean Yellow-legged Gulls, are similarly related.
- (3) The Lesser Black-backed Gulls *L. fuscus* from The Netherlands are indeed *graellsii*, being closely related to *graellsii* from other locations.

taxa *taimyrensis* and *beuglini* being closely related. Statistically, this tree is, however, not very robust (the only branch points that are more than 90% significant are the clustering of *argenteus* with *argentatus* and of *atlantis* with *michabellis*, and the splitting of Common Gulls from the rest of the gull assemblage). It would appear, therefore, that these taxa are very closely related to each other.

The AFLP analysis also revealed that all these gull taxa were much more inbred than would be expected by chance. This is perhaps not surprising, for all taxa show great breeding-site fidelity, which will tend to pair up individuals with genetic relatives. It was also found that there are high levels of gene flow among taxa. This apparently contradictory finding may simply be a result of the recent massive expansion of gull populations, associated with human activity. Studies by other workers (Johnson 1985; Snell 1991) have also suggested that different gull taxa are very closely related, with a high level of inbreeding. The phylogenetic history of the gulls would appear to be extremely dynamic, with rapid radiation from a common ancestor, and inbreeding caused by breeding-site fidelity.

Conclusions and comment

The research by de Knijff *et al.* used a method of genetic analysis which avoids many of the pitfalls associated with the techniques employed by other researchers. Nevertheless, although their study has provided a great deal of useful new information, it has not solved the problem of understanding the relationships among all these gulls. Most of the observable genetic difference between any two individual gulls, for example a *cachinnans* and a *graellsii*, is due to the genetic variation within these (sub)species, rather than the genetic variation between the two taxa.

It is clear that the easily visible phenotypic differences among 'classic' individuals of, for example, *fuscus* Lesser Black-backed Gulls, *michabellis* or *cachinnans* 'Yellow-legged' Gulls and *argenteus* or *argentatus* Herring Gulls do not, according to this study, correspond with an equally clear-cut hierarchy of genetic differences. How is this possible? Two explanations are proposed. One

option is that, although, following splitting of ancestral gull species during the glaciations, the process of speciation among the Western Palearctic large gulls has begun, many of the taxa are still in the 'grey zone' (Collinson 2001), whereby it is not possible to show that all the criteria for full species status have been achieved. Specific changes in display, behaviour and breeding biology have created near-complete reproductive isolation of some of the taxa, and on a relatively short timescale (Brown 1967; Yésou 1991); but the lineages have not been split for sufficiently long for nuclear-DNA sequences to have diverged clearly.

The second option is to assume that all these gulls belong to one species which radiated out of the Caspian area after the Ice Ages as a chain of small founder populations, evolving and changing as they did so. Subsequent hybridisation among the expanding descendants of these founder populations has created the enormously variable range of gulls which we see in Eurasia today. This second model is analogous to the repopulation of Europe by Man after the Ice Ages.

Neither explanation necessarily excludes the other completely, and neither can be discounted on the basis of these genetic results. De Knijff *et al.* favour a combination of the two.

In 1993, *British Birds* adopted the policy of giving species status to some of the forms of Herring Gull (*Brit. Birds* 86: 1-2), recognising 'Yellow-legged Gull' as a full species, *Larus cachinnans*, with western and eastern subspecies *L. c. michabellis* and *L. c. cachinnans*. Although this represented a consensus view among European birdwatchers at the time, such a split was also criticised as being premature, with much of the biology and identification criteria of these birds remaining insufficiently documented (*Brit. Birds* 86: 316-319). In fact, de Knijff *et al.* do not recommend wholesale changes to the taxonomic arrangement of 'herring gulls', since their analyses revealed no clear emerging pattern to distinguish whether there are two, three or seven species in this complex. Nevertheless, their work, when taken together with previous analyses of the evolutionary history of these gulls (Mayr 1963; Barth 1975), does suggest that 'Caspian Gulls' *L. c. cachinnans* are a basal group of

the other European large white-headed gulls, and that 'Yellow-legged Gull' as recognised by *British Birds* is consequently a paraphyletic grouping, since some of the populations that are descended from the common ancestor of *cachinnans* and *micrabellis* have been excluded from the species.

While we must be aware of the limitations of the data discussed above, and avoid over-interpretation, it does seem that the current *British Birds* treatment of 'Yellow-legged Gull' (i.e. the lumping of the taxa *micrabellis* and *cachinnans*) is untenable.

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Appendix

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique produces a 'genetic fingerprint' from individuals of species or subspecies, and has the advantage that it does not require any of the specific DNA sequence of the taxa to be known already. Nuclear DNA is cut into fragments, using restriction enzymes; oligonucleotide adaptors of known sequence are then ligated to the cut ends of the DNA. PCR (polymerase chain reaction) amplification is performed using primers the 5' sequence of which is complementary to the adaptors, but the 3' sequence of which overruns into the nuclear DNA. PCR products are, therefore, produced only from that minority of nuclear restriction fragments having ends that are complementary to the 3' ends of the PCR primers. Up to 150 nuclear sequences of different sizes, produced by the PCR reaction, can then be separated by electrophoresis, visualised as bands in a gel: the genetic fingerprint of the individual. Presence or absence of bands of all sizes can be scored to provide a measure of the difference in genetic sequence among individuals of the same or of different taxa.

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EDITORIAL COMMENT Although Martin Collinson's paper suggests that the present treatment of the Western Palearctic large white-headed gulls by *British Birds* may be inappropriate, we intend to refrain from making a further change to our accepted list of species until the BOURC reaches a decision about the treatment of these forms. We look forward to any such decision with great interest.