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PERSPECTIVE

SCIENTIFIC CONTROVERSY OVER AVIAN TAXONOMIC CHANGES, BASED ON DNA HYBRIDISATION

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ABSTRACT. - The long-standing classification of birds has been greatly modified by work on DNA hybridisation, changes which have rapidly been picked up by the popular literature. Some aspects of the work as well as the results have been controversial. Reasons for such controversies are discussed, and it is pointed out that moderating opinions now permit a more rational assessment of each of the proposed changes. There are still major discrepancies between fossil and DNA evidence which require explanation. A danger of confusion exists between changes in higher level classification, a narrowing of the species concept, and a change in the species concept, all trends that are being absorbed into the literature simultaneously.

KEYWORDS. - DNA hybridisation, taxonomy, Aves, birds, species concepts.

INTRODUCTION

Taxonomy is often regarded by university students, and by many professionals, as one of the most boring and least necessary areas of biology. Yet the grouping and naming of organisms generates heated controversy. Like politics and religion, it seems, the less the certainty of absolute answers, the more stubbornly do people stick to their opinions. Periodically, and at least as often as in other biological disciplines, taxonomy is swept by a tide of new techniques that washes away large embankments of established order, in spite of efforts to shore them up with breakwaters and barricades, and deposits them on a coastline of new opinion. Other biologists then begin to explore the hinterland, until the next tide comes along and swamps their boats.

The transience of taxonomic opinion is well demonstrated by ornithology. A textbook written in 1938 would likely begin with passerines and end with galliformes. One written in 1968 would probably begin with the ostrich and end with the crows. One written in 1998 would,

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perhaps as likely as not, begin with the galliformes and end with the sparrows. This fluidity extends not just to the sequence of orders and families, but to the recognition and naming of species in both English and scientific terms, and the ranking and recognition of all categories in the taxonomic hierarchy.

Taxonomic change is as active as ever in ornithology, where there have been two recent main challenges to formerly established opinion, one at the species level and one at the higher levels of classification. Both these challenges involve methodology as well as the philosophical basis of taxonomy. The challenge at species level is between the so-called biological species concept and the newer phylogenetic species concept. The challenge at the higher levels is posed by the way in which DNA hybridisation methods have been applied.

HISTORICAL BACKGROUND

The Wetmore sequence

Since the 1940s, the sequence of listing families and higher categories of birds has been the one proposed by Wetmore (1940). This was set out de novo as a linear sequence, not derived from the construction of cladograms or other branching patterns. Wetmore's (1930) original paper gave little discussion for each family or order, and at this date it is a matter for speculation as to how much Wetmore's opinions were influenced by his own work (mainly on palaeontology and anatomy) and how much by his accumulation of the knowledge and opinions of other ornithologists, especially the anatomical work by Beddard (1898) and Gadow (1893).

The adoption of Wetmore's sequence by the ornithological community was not immediate nor universal. It was adopted quickly in the United States and parts of Europe, slowly in the United Kingdom, and with two main variations: listing the families within the Passeriformes with the crows (Corvidae) first or early, or listing them with the crows last. A 'crows last' sequence was adopted by major early works, and in the Southeast Asian region by Smythies (1953, 1960); a 'crows early' sequence has been common to regional texts such as King et al. (1975), Ripley (1982) and Lekagul & Round (1991). As a result of its general adoption, the Wetmore sequence is the one most familiar to most ornithologists. It has not been a static sequence, as various modifications were made preiodically by Wetmore (1934, 1960).

World lists based on the Wetmore sequence have all begun with the ratites (ostriches, emus, etc.). As will be seen below, this has had methodological repercussions on the most recent classifications even when they reject the Wetmore sequence.

Biochemical trends

Beginning in the 1960s, biochemistry began to play an increasing part in classification. For example Mainardi (1963) used immunological distance to plot the relationships of birds within the order Galliformes. Immunological distance gave a single number to compare between pairs of species, and could be expressed as distance on paper so as to produce a net-like or web-like diagram.

Sibley & Ahlquist (1970, 1972) used eggwhite proteins as a source of taxonomic information, using electrophoresis to make comparisons between the patterns of closely and distantly related birds. This method made visual comparisons easy, especially as regards the presence or absence of particular proteins, and allowed a degree of quantification such as distance and rate moved through the electrophoretic gel.

Subsequently, various biochemical methods of increasing sophistication have been developed that have worked down towards the gene level. Since most taxonomists intend that their classifications should reflect evolutionary relationships, and since these should reflect genetic similarity, the trend has been to concentrate increasingly on those molecular techniques which reveal information about DNA. One of these is DNA hybridisation, of which the following discussion is based entirely on Lewin (1988a, 1988b) and Sibley & Ahlquist (1990).

Primate DNA

Although recent discussions have centred around birds, an early source of controversy was work on the relationships of humans *Homo sapiens*, gorillas *Gorilla gorilla*, and chimpanzees *Pan troglodytes*. In the 1960s it was shown that all three are genetically very closely related, sharing perhaps 98% of their apparent genetic content (Ciochon & Fleagle 1987). The intractable taxonomic problem was to break down this trichotomy into two dichotomies by demonstrating which two of the three forms are most closely related.

An answer was announced by Sibley & Ahlquist (1984) who reported, from DNA hybridisation experiments, that humans and chimpanzees are each other's closest living relatives, and that gorillas diverged earlier. This was an unexpected result, for the conventional view had been that humans had diverged earlier, with gorillas being more closely related to chimpanzees. Other biochemical and molecular techniques were then applied to the question, including protein electrophoresis, DNA restriction mapping and DNA sequence analysis, as well as conventional anatomical analysis. Sibley & Ahlquist (1987) were able to publish a much larger set of DNA hybridisation results, confirming their earlier finding.

The controversy arose through conflicting results and opinions of other researchers which were then offered for publication. These criticised (a) the conclusions reached by Sibley and Ahlquist, and (b) their method of analysis and data handling. To an observer outside the conflict, the controversy seems to have been heightened by personal animosities and preconceived opinions. There are, however, some real scientific issues for consideration. These are the value of the DNA hybridization technique; the choice of statistical analysis of the raw data; the availability of raw data to other scientists; and the question of data manipulation.

As an aside to the question of primate relationships, work on the DNA of orang utans has suggested that the degree of difference between the Bornean and Sumatran populations of orang utans *Pongo pygmaeus* is equivalent to that typically found between pairs of species, not subspecies. The conclusion would be that two species of orang utans should be recognized. Similarly, the degree of difference between Sumatran and mainland Asian tigers *Panthera tigris* has recently led to the suggestion that Sumatran tigers should be recognized as a full species. This brings in two further points: the choice of a standard, objective or mechanical, arbitrary degree of difference for each level of the taxonomic hierarchy (subspecies, species, genus, etc.), and the selection of information to suit the more practical or political purposes of conservation. Both points are discussed further below.

DNA HYBRIDISATION TECHNIQUE

Laboratory method

The essence of DNA hybridisation is to compare the similarity of DNA strands between any two species. The more similar they are, the smaller is the evolutionary distance and the closer the relatedness between the two.

DNA is taken from one of the species to be tested, cut into lengths of about 500 base pairs, and separated from double strand molecules into single strands. Those sequences that appear in multiple repeats are removed. This theoretically leaves behind only unique sequence DNA, and involves a decision about the significance of the 'redundant' repeated DNA sequences that are typical of all organisms. Their removal is in effect a statement that they are not significant, but this is potentially in conflict with the phenetic approach or assessment of overall similarity that is the basis of the DNA comparison.

These single strands of DNA are made radioactive, and are then known as tracer DNA. They are added to a large excess of single stranded DNA from the second species, known as the driver DNA. The mixture is allowed to anneal, during which strands of tracer DNA will form double strands (or hybridize) with strands of driver DNA where their sequences are sufficiently similar. The more similar they are, the more tightly will they hybridize.

The duplex formed during annealing is then subjected to gradually increasing temperatures, from about 60°C to 90°C. This progressively splits the double stranded fragments apart, and this is measured by the number of radioactive counts lost at each step in temperature. The result is a graph, showing as a curve the increasing percentage of single-stranded DNA against increasing temperature.

If two samples of DNA are taken from the same species, then hybridisation between tracer and driver DNA should be complete. High temperatures would be required to release all of the radioactive tracers. If the DNA is from two different but closely related species, hybridization would be less complete, and lower temperatures would suffice. If the DNA is from two distantly related species, still lower temperatures would be adequate. The degree of lowering of the 'melting' temperature, between a same-species sample and a different-species sample, is taken to indicate the initial degree of hybridization, and to be a measure of evolutionary distance.

In practice, even if tracer and driver DNA are from the same species, duplex formation ('hybridisation') is never complete. It is still an open question as to whether this unhybridised proportion of DNA contains valuable but inaccessible information, misleading information, or merely repetitious information. Further points of discussion have been the experimental procedure itself (such as the preparation of the DNA), the conditions under which the experiments are usually done (influencing the kinetics of the initial duplex formation), and the kinetics of the subsequent melting.

Choice of statistical analysis

The above uncertainties will have different effects on results, depending on the statistical analysis performed. Sibley and Ahlquist (1990) plot data cumulatively to give a sigmoid curve showing the melting profile for the entire DNA sample. Two such curves (one for

tracer and driver DNA from the same species, the other for tracer and driver DNA from the two compared species) are plotted, and the difference in temperature at which exactly 50% of the DNA has dissociated in each of the samples is the critical measurement. This is the ΔT50H statistic.

Proponents of other methods may prefer to exclude the initial difference in hybridisation between the two samples, and take the difference in median temperature at which 50% of the hybridised DNA (only) has dissociated in each of the samples. This is the Tmedian or Tm statistic. A third method is to plot a bell-shaped curve showing the temperature step which is responsible for the greatest degree of dissociation. This, the Tmode, was the statistic first used by Sibley and Ahlquist.

Sarich et al. (1989) assessed the role of DNA hybridisation as a guide to phylogeny, and strongly favoured the Tmode statistic, in opposition to Sibley and Ahlquist.

Although there has been intense debate over the use of these different statistics, recent work has not been able to substantiate any strong theoretical arguments for or against any one statistic over the others. Data produced by any of the methods appear to be highly correlated with data from the other methods.

Data manipulation

A more substantial criticism of the work by Sibley and Ahlquist concerns corrections to their raw data. Marks et al. (1989) used about 10% of the human/chimpanzee/gorilla data (all that was available to them at the time) to calculate for themselves the ΔT50H statistic, and compared these with the same measures published by Sibley and Ahlquist. They found that 40% of the measures were significantly different, by more than 0.5°C in a range of 1°C to 3°C. Marks *et al.* (1989) suggested that up to 40% of the data concerning humans and apes may have been subjected to manipulations of an unspecified nature. This seems to have been confirmed by an independent analysis of the full data set (Lewin 1988b).

Ahlquist has stated (Lewin 1988b) that corrections to the data are derived from long experience in examining melting curves, and Sibley that the errors corrected had seemed to be clear and in an easily detected direction. Nevertheless, it is still the case that the ordinary reader cannot detect which results have been altered and which not; what is the proportion of altered to unaltered results; and what criteria were used to determine whether alteration was justified in any given instance. So far as the work on bird DNA is concerned, the nature of the corrections has been described by Sibley & Ahlquist (1990) and their proportion estimated as 20% of the total, but it is again not possible to determine which are the corrected and which the uncorrected results.

AVIAN DNA

Data set, matrix formation and data pooling

About 26,554 DNA hybridisation experiments were performed on 1,069 species of birds by Sibley & Ahlquist (1990), compared with 514 experiments on three species of humans and apes. Per species, the data for humanoid apes are therefore much fuller than the data for birds. The bird data or 'Tapestry' were first displayed in the form of a fifty-foot long poster

at the International Ornithological Congress in Ottawa in 1986, and are shown by Sibley & Ahlquist (1990) as about 30 separate dendrograms.

There is variation between individuals in a species, so that the melting point difference will give differing results if an individual of species A is compared one by one with several different individuals of species B. Experiments on species A and B will giving differing results from time to time depending on the size distribution of the fragments of tracer DNA (which cannot be exactly 500 base–pairs in length, but shows a scatter of lengths), and presumably depending also on the scission points in the tracer DNA.

Points such as these will have a bearing on how reliably the results from the DNA hybridisation melting curves can be transformed into a dendrogram. The distance data are first arranged into a matrix. Ideally, matrices are composed only of comparisons among single species, used both as drivers and tracers (for definition, see section on Laboratory Method). In practise, many of the cells in the matrices compiled by Sibley & Ahlquist (1990) include measurements from different species "if the data show they can be pooled". Pooling of several species into the same cell of a matrix is appropriate if the species are members of a monophyletic lineage and the rates of genome evolution are identical or nearly so (Sibley & Ahlquist 1990).

What are the implications of such pooling? As evolution proceeds by dichotomies, all the species in one clade ought to show more similar DNA melting curves than any shows when compared with a member of another clade. In reality, this is not the case. An example can be drawn from Ahlquist et al. (1984), in which the Bornean Bristlehead *Pityriasis gymnocephala* is compared with 66 other species of birds. Those showing the least difference from *Pityriasis* in their melting curves are the Corvidae (Tribe Cracticini) and the Corvidae (Tribe Oriolini), using the classification of Sibley & Ahlquist (1990). The five species of Cracticini show ΔT50H between 3.9 and 5.0, while the 14 species of Orioloni show ΔT50H between 4.5 and 5.9. Clearly these data show a substantial degree of overlap, and if more species of Cracticini had been available for comparison the degree of overlap might have been still greater. It then becomes partly a matter of opinion and partly a decision of science, as to whether one is willing to accept the averaged data as demonstrating a closer relationship between *Pityriasis* and Cracticini than between *Pityriasis* and Oriolini. Averaging the numbers would certainly indicate this, but averaging might conceal other problems. Specifically, the Sibley & Monroe (1990) concept of the Cracticini includes the traditionally separate Cracticidae and Artamidae, and their concept of the Oriolini includes the Oriolidae and Campephagidae. Therefore each of these groups is conceivably not monophyletic, which would invalidate the wisdom of averaging.

Linking *Pityriasis* with the Cracticini at this decision-making level is therefore dependent on previous layers of decision-making in other parts of the taxonomic hierarchy (or DNA matrix). No decision stands independent of other decisions, each of which contains value judgements as well as scientific judgements. If the prior decision to link Cracticidae and Artamidae had been at fault, then the decision to link *Pityriasis* with the newly defined taxon Cracticini would be influenced by this fault. In fact, in this example linking *Pityriasis* with the members of the previously recognized Cracticidae seems well founded and agrees with a range of other evidence on the species, but the composition of both the Cracticini and Oriolini as defined by Sibley & Ahlquist (1990; see also Sibley & Monroe 1990) are unusual, and to subsume them within a greatly expanded Corvidae is one of Sibley and Ahlquist's more dramatic hypotheses. This relates back to the judgement on whether taxa are or are not members of a monophyletic lineage, and therefore as to whether the data can be pooled, as

well as a quite separate judgement of the rates of genome evolution in the different birds tested.

As mentioned above, more than 26,000 crosswise tests on pairs of species were performed by Sibley & Ahlquist (1990). However, with a total of about 9,600 recognised bird species in the world, the result is that less than 4% of the possible species pair comparisons have been made. In other words, the data set is very fragmentary. To a great extent this does not matter, as the strength of the work is in the clarification of patterns at the family level and above. For example, in assessing the relatedness of several species of ducks, it is clearly unnecessary to make species by species comparisons between each of the ducks and a woodpecker. On the other hand, the detection of unexpected phylogenetic relationships must be limited by imagination in making what one would have thought to be unnecessary (and therefore untested) comparisons. Furthermore, there is evidence that even at the family level the matrix is incomplete (Peterson 1992). This, and the limitations on the availability of DNA material from desirable species, place limits on the significance and usefulness of the data matrix. Possibly the form of publication, in massive, comprehensive volumes, has helped to create the idea that Sibley and Ahlquist were hoping to provide the last word on many of these issues, rather than producing hypotheses in need of further testing.

From matrix to dendrogram

The completeness of the data set mentioned above, whatever the limitations it places on species comparisons, has an effect on the success in converting the numerical data into branching trees. Sibley & Ahlquist (1990) used a manual implementation of an unweighted pair-group method using arithmetic averages (UPGMA). Using incomplete matrices means that the trees constructed are of simple ladder structure. Structures other than ladders occur only in results from those experiments where taxa were radioactively labelled. Only 308 taxa were so labelled, and only these taxa can therefore be used in calculating the internal branching structure of the dendrogram. The choice of these 308 taxa (out of 1,069 species tested, and 9,600 birds in the world) must influence the reconstruction of these branches.

Peterson (1992) has also shown that because of the incompleteness of the matrix, the choice of the tracer taxon determines the structure of the dendrogram. If only a basal lineage in a clade is radioactively labelled, then either the relationships of the remaining taxa are unresolved, or they will resolve into a ladder with short branches which represent noise in the data. The tracer taxon will always be placed at the highest resolved node in the tree, regardless of its true position. Based on this, Peterson has suggested that the matrix of family comparisons was as incomplete as that of species comparisons, so that the analyses were insufficient to complete the data matrix even for family level comparisons of birds.

The method presented by Fitch & Margoliash (1967) was used by Sibley & Ahlquist (1990) to construct their dendrograms. All the comparisons they made were between the homoduplex curve and the heteroduplex curve, not between heteroduplex curves. That is to say, the comparisons were between a "Species A x Species A" melting curve and a "Species A x Species B" melting curve, not between (for example) a "Species A x Species B" versus a "Species A x Species C" melting curve. Heteroduplex curves equidistant from the homoduplex may be closely related to each other, if they are on the same branch of the tree, or they may be on different branches yet equidistant from the homoduplex. Once again this involves a judgement of monophyly, which in turn depends on a chain or web of previous decision-making, which fits with the appellation of the matrix as a 'Tapestry'. The Fitch–Margoliash

trees are presented as unrooted networks, without any specifically stated outgroup for comparison (Peterson 1992). The trees do not appear to have been rooted even at the mid point of their longest branch, a method which would have been consistent with the assumption of a molecular clock (i.e., a steady rate of genome evolution as postulated).

It is perhaps therefore inevitable that there are conflicts between the constructed Fitch-Margoliash trees and the Tapestry, including conflicts that arise from the lack of a stated outgroup, hence the lack of a specified rooting point for the trees, and the averaging and pooling process used in constructing the matrix. Where such conflicts occur, Sibley and Ahlquist favour the Tapestry rather than the Fitch-Margoliash tree. They suggest that differences in the rate of genome evolution account for some of the discrepancies, and discuss generation time as a source of such variation. Often, little information is available for any given case. For instance, the isolated position of the buttonquails Turnicidae may be influenced by a high rate of genome evolution, and Sibley & Ahlquist (1990) cite a short generation time, three to five months in captive birds, but also state that generation time in the wild might be a year, i.e., not significantly different from small members of the Phasianidae or Thinocoridae. Other possible influences on the rate of genome evolution, such as natural selection, sex ratio, breeding system, and the influence of dispersal characteristics, are not considered (Peterson 1992). The idea of a DNA clock does not mean that all sections of the DNA strand have undergone change at the same rate, and it would be difficult to know whether the particular fragment of DNA isolated to work on is part of a gene or genes that has evolved more rapidly than other fragments.

Dendrogram to linear sequence

Lists of taxa are one–dimensional arrangements of the results from a multi–dimensional evolutionary process. Once dendrograms have been constructed, a list can then be written by reading off the names of taxa along the ends of each branch in turn.

This is more easily said than done, because any dendrogram, and any constituent part of any dendrogram, can in principle be flipped through 180 degrees without disturbing its branching pattern. Both small and large chunks of a list can therefore be reversed without any implications for or from the underlying data. Seeing two taxa listed consecutively in a linear sequence conveys rather little information about their degree of relatedness. Perhaps the most glaring example of this in the work of Sibley & Ahlquist (1990) and Sibley & Monroe (1990) is the juxtaposition of the storm-petrels and the New Zealand bush-wrens.

Sibley & Monroe (1990) have provided a world list of bird species, in a classification based primarily on the evidence of relationships from comparison of their DNAs (Sibley & Ahlquist 1990). Both Sibley & Monroe and Sibly & Ahlquist refer to this repeatedly as evidence of phylogenetic relationships. However, the analysis is definitively not phylogenetic in the narrow sense of this term.

Hennig (1966) introduced cladistics as a formal method of classification in which the shared characteristics of organisms were distinguished into two types: those characteristics shared with other organisms because they are ancestral to a larger pool. For example monkeys and frogs both have four limbs, not because monkeys and frogs are most closely related to each other but because four limbs are an ancestral character for all members of the group Tetrapoda (amphibians, reptiles, birds, mammals). The possession of four limbs is a shared primitive characteristic or plesiomorphy for monkeys and for frogs, and not relevant to branching

points separating those two animals. Horses, donkeys, tapirs and rhinoceroses all have an enlarged weight-bearing third digit in the foot, a condition thought to have evolved only once in mammals and showing that all these mammals are members of one branch of a tree: the condition is a shared derived characteristic or apomorphy.

The analysis of apomorphies, and the rejection of plesiomorphies, results in a cladistic analysis which is phylogenetic in the strict sense. DNA hybridisation is incapable of distinguishing between primitive and derived shared characteristics, and lumps all sharedness within a single measurement. It measures overall similarity, and is therefore a phenetic method of classification. If rates of genome evolution are not steady, differences will result between a phenetic and a phylogenetic interpretation. Thus if a lineage splits into A and B, through one difference arising, and lineage B then splits into B and C through two differences arising (note, the rate of genomic evolution has altered), then A and B will be distinguished by only one difference, but B and C will be distinguished by two differences. A cladistic, phylogenetic analysis should correctly interpret the sequence of splits, but a phenetic analysis would place the split between B and C prior to the split between A and B.

Such differences would show their impacts in the linear classification of Sibley & Monroe (1990) at the higher taxonomic levels of family and order. The sequence and recognition of species in the list should hardly be affected, because species level taxonomy was not investigated by Sibley & Ahlquist (1990). The recognition of species by Sibley & Monroe (1990) is therefore separate from the DNA evidence, and is based on other work. Almost all of the taxonomic arrangement below the level of the Tribe is based on traditional arrangements. There are some exceptions, including new groupings at and below the level of the traditional family, where either nodes (branches in the Sibley and Ahlquist dendgrograms) have not been given names by Sibley and Monroe, or where names given by Sibley and Monroe are not supported by the existence of a node in a dendrogram. Peterson & Stott (1992) give many examples. The Cariamida (South American seriamas) and the Stercorariini (skuas) are not supported by nodes according to the DNA data, and nodes equating to the traditional Alcedinidae (kingfishers) and to a split between *Nyctiornis* and other bee-eaters (Meropidae) are not recognised by any name in the classification. Thus there are many inconsistencies between the branching data and the naming of branches. There are also many cases of inconsistency in the recognition of superspecies and subspecies groups (Peterson & Stott 1992), while changes at species level are often based on personal communications of opinion by a single contact person, not based on rigorous analyses either of literature or of museum specimens. On the contrary, the Wetmore-based classification of the Peters checklist of birds of the world, though carried out over such a long period that there were significant changes in species concepts, was at species level based on detailed museum analysis of many thousands of specimens by many researchers (e.g. Peters 1934, Mayr & Paynter 1964).

Of concern is the amount of reasoning behind taxonomic decisions (Sibley & Monroe 1990). While stating that amongst the Spiderhunters, Nectariniidae, the putative species *Arachnothera everetti* may be conspecific with *A. affinis*, no space is available either to state the evidence or to adduce its significance. Only subsequent treatments based on Sibley & Monroe (e.g., Inskipp *et al.* 1996) have been able to compile evidence, which thus becomes a form of *a posteriori* reasoning. This point is quite minor in comparison with some unreasoned changes introduced into the original classification. Sibley & Ahlquist (1990) moved the genus *Culicicapa* from the Old World Muscicapidae to the Australasian Eopsaltridae, based on DNA hybridisation data. Sibley & Monroe (1990) gave the compatible listing. But *Culicicapa*

was moved back to its traditional place with the flycatchers by Sibley & Monroe (1993). No reason was given, leaving the reader and user to conclude either that the original experimenters no longer trust their DNA data, or that they or other authors have performed additional work, either published or unpublished but not referenced and therefore of uncertain accessibility, or indeed that there is no reason for the change other than a gut feeling on the part of the authors. Although the small number of species involved may suggest that this is an insignificant case, the lack of overt reasoning seems to be a blow at the more general credibility of the work.

Another example of significant change in taxonomic position, within the opinions of DNA hybridisation workers, is of the Parvclass Galloanserae (Table 1). Sibley *et al.* (1988) placed this group (the Galliformes and Anseriformes) together with the ratites as members of the infraclass Eoaves; Sibley & Monroe (1990) placed them in the Neoaves. This is a shift between categories at a very high taxonomic level, and therefore a question which one might have expected to have been resolved definitively at a much earlier phase in an 11.5 year study (Sibley & Ahlquist 1990).

Table 1. Summary classification of birds, derived from Sibley & Ahlquist (1990).

Class Aves
Subclass Archaeornithes
Subclass Neornithes
Infraclass Eoaves
Parvclass Ratitae
Infraclass Neoaves
Parvclass Galloanserae
Parvclass Turnicae
Parvclass Picae
Parvclass Coraciae
Parvclass Coliae
Parvclass Passerae

Major DNA insights

The DNA hybridisation studies have confirmed many of the traditional taxonomic arrangements based on anatomy and morphology (Gadow 1893, Wetmore 1940). They have also demonstrated the role of convergent and adaptive radiation in ecology and morphology, some of it confirming or being confirmed by independent work (Bledsoe 1988, Prum 1988, Lanyon & Hall 1994). The summary classification of Sibley & Ahlquist (1990) contains surprises both in the groups recognised and their relationships to one another (Table 1).

Of special significance is the recognition by Sibley & Ahlquist (1990) of a major corvine radiation (Corvidae), mentioned above in relation to *Pityriasis*. The enlarged concept of this family includes the traditional families of birds of paradise (Paradiseidae), drongos (Dicruridae), orioles (Oriolidae), monarch flycatchers (either Monarchidae or part of the Muscicapidae), butcherbirds (Cracticidae), Australian sittellas (Neosittidae) and other groups. The inclusion of some of these groups is controversial, of others not, and its main significance at present may be as a hypothesis for the more detailed testing of relationships according to DNA sequencing and other refined techniques.

Another important set of hypotheses relates to the Australian passerines. Groups that had been thought related to Asian or European birds appear now to be more closely related to other Australian groups, so that songbird radiation on the isolated Australian landmass is equivalent to, but at a lower taxonomic level than, that of the marsupials (Sibley & Ahlquist 1985).

Another example of a radical departure from traditional taxonomy is the grouping of the traditional groups of storks (Ciconiidae), grebes (Podicipediformes), waders (Charadriiformes), birds of prey (Accipitriformes), divers (Gaviiformes), penguins (Sphenisciformes) and others within a single greatly expanded order, Ciconiiformes, and that this is placed within a superorder Passerimorphae together with the pigeons (Columbiformes), cranes (Gruiformes) and passerines (Passeriformes). In other words, albatrosses and petrels are thought more closely related to pittas and sparrows than either is to woodpeckers, rollers or hornbills (Table 2). This seems to conflict with a great deal of morphological and anatomical evidence, which in particular supports a closer relationship between woodpeckers and passerines (Feduccia 1996), and is bound to require repeated testing of results.

Table 2. Summary classification of the Passerae in relation to other Parvclasses of birds, derived from Sibley & Ahlquist (1990).

Parvclass Galloanserae
Parvclass Turnicae
Parvclass Picae
Parvclass Coraciae
Parvclass Coliae
Parvclass Passerae
Superorder Cuculimorphae (cuckoos)
Superorder Psittacomorphae (parrots)
Superorder Apodimorphae (swifts, hummingbirds)
Superorder Strigimorphae (owls, nightjars)
Superorder Passerimorphae
Order Columbiformes (pigeons)
Order Gruiformes (cranes)
Order Ciconiiformes (birds of prey, pelicans, penguins)
Order Passeriformes (songbirds)

Osteology has assessed the tropicbirds and frigatebirds as primitive within the traditional order Pelecaniformes, and viewed pelicans and cormorants as derived and specialised (Lanham 1947; Olson 1977). DNA hybridisation data (Sibley & Ahlquist 1990) and DNA sequence data (Hodges & Sibley 1994) differ strongly, making the pelicans and the shoebill close relatives, placing frigatebirds together with penguins, divers and shearwaters, and making tropicbirds the isolated relicts of an ancient divergence. Such basic differences of opinion are unlikely to be resolved without much more critical step-by-step analysis and, one suspects, neither position is likely to prove fully correct.

Fossil Evidence

Some of the most interesting evolutionary events at the higher levels of avian taxonomy can be addressed by the study of fossils. Fossils represent an independent line of enquiry which can be used to test the reasonableness of DNA hybridisation results.

Many of the smaller discrepancies between the UPGMA derived Tapestry and the branching patterns of Fitch-Margoliash trees are attributed to differences in the rates of genome evolution (Sibley & Ahlquist 1990). However, the concept of a fixed (or nearly fixed) molecular clock is basic to the theory behind DNA hybridisation experiments, that the longer two groups have been separate the greater the DNA disparity between them. Sibley and Ahlquist assume that most modern bird orders diverged well back into the Cretaceous era, and take the assumed origin of ratites (ostriches, emus, etc.) at that time as an underlying measurement of the rate of the molecular clock. A contrary view based on fossils is that there was an explosive divergence of birds in the early Tertiary, in which most modern orders arose within about 10 million years (Feduccia 1996). This view would contradict the assumed rate of the molecular clock, remove the ratites as pivotal in determining that rate, and make it difficult for DNA hybridisation — or any other, perhaps cladistic, method — to interpret the sequence of very tight clustering of the major branches. However, this view is also not held by all palaeontologists; for example, work by Boles (1997) suggests the diversification of birds before the Tertiary, more in agreement with the Sibley and Ahlquist timing of events.

The phylogeny of the ratites has been one of the most discussed of all avian taxonomic questions (Huxley 1867, Cracraft 1974, Olson 1985). There are two views: that living ratites are a monophyletic group, of ancient origin, split by continental drift (Cracraft 1974), and that they are the relicts of independent evolutionary radiations from an earlier range of flighted palaeognathous birds (Houde & Olson 1981). Sibley & Ahlquist (1990) found the living ratites to be monophyletic. Current information is probably inadequate to make a decision between these hypotheses. The assumption of a Cretaceous origin within the then united southern continents neatly explains the existing morphological similarities and geographical distribution of modern ratites, but is unsupported by fossils of the critical age (the earliest fossils of flightless ratites date from 30 million years after the split between the southern continents: Feduccia 1996). The assumption of separate origins of modern flightless ratites from within a group of northern flighted lithornithid ancestors ties the fossil evidence neatly together but ignores biochemical evidence and has to assume that flight persisted long enough to explain modern distributions.

As Sibley & Ahlquist (1990) use the split between ratites of the southern continents during the Cretaceous as a criterion for measuring the rate of genome evolution in ratites, doubts about the origins of ratites (especially its timing in relation to the origin of other avian orders) could have serious implications for their construction of dendrograms.

CONFLICTS IN CONCEPTS

The narrowed view of species limits that is adopted by Sibley & Monroe (1990) is part of a general trend of narrowing species limits amongst ornithologists. This has become mingled, and is in danger of being confused, with a recent shift towards a new phylogenetic species concept (PSC) (Cracraft 1983, Hazevoet 1995), and away from the biological species concept (BSC) promulgated by Mayr. Under the PSC, a species is the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent. Under the BSC, a species is a group of interbreeding natural populations that are reproductively isolated from other such groups. Part of the difference between the two is that the PSC emphasises intergenerational genetic continuity (vertical patterns of descent), whereas the BSC emphasises intra-population genetic panmixia, or the horizontal (in time) genetic cohesiveness as well as distinctness of a population. This is part of an academic

discussion that ought not to be confused with the controversies of DNA hybridisation, yet is becoming associated with it through a distinct process. This process is the adoption of both the Sibley, Ahlquist and Monroe taxonomy, and the phylogenetic species concept, within the popular as well as scientific ornithological literature. Thus a phenetic classification at the higher taxonomic level is becoming a vehicle for a species concept which is overtly not phenetic. These two approaches are not methodologically congruent, but their fusion has been facilitated by the narrower concept of species that both have adopted.

Inskipp et al. (1996) have published a recent checklist for the Oriental region, employing the Sibley & Monroe (1990) sequence, and the biological species concept. The latter is evinced through their summaries of evidence for species limits, which accept overlap and evidence of suppressed levels of hybridisation at parapatric boundaries, as criteria for recognising species. Significant differences in voice and plumage are likewise accepted as criteria for species, but here the amount of difference that is accepted as significant is subject to opinion and consensus. Collar (1997) has reviewed the rôle which birdwatchers therefore play in detecting differences between taxa, and in the process of taxonomic acceptance of species. Geographical differences in voice are a topic to which birdwatchers have contributed a great deal, although the significance of the variation is not well understood.

Mayr & Bock (1994) have stressed the distinction between provisional classifications and standard listings. A provisional classification is a vehicle for the results of phylogenetic analyses, which represent hypotheses for further investigation. They may change rapidly and are foci for controversy and study. Standard listings are classifications in a generally accepted sequence, which serve as vehicles of communication, and therefore require greater consistency. A standard sequence does not need to agree in all respects with the latest phylogenetic hypotheses, but may be changed only when the level of agreement amongst macrosystematists has reached such a level that change is generally accepted. Who these systematists would be, and how consensus might be reached, are unanswered questions, although the International Ornithological Congress has long been proposed as a body suitable to make decisions on sequences and lists (Lack 1967)

Thus Mayr and Bock stress the difference between hypothesis and practicality. In this context the rapid adoption of the Sibley & Ahlquist (1990) and Sibley & Monroe (1990) is a cause for concern, because it has begun working into the popular and semi-scientific literature prior to rigorous testing. This argument is quite independent of the merits of the whole or any particular part of the DNA hybridisation work. The significance of this argument is considered slight by Inskipp et al. (1996), but without going into the detail of each point presented by Mayr and Bock.

Practicality is also stated as an advantage of the PSC, in that it gives fairer attention to geographical isolates for conservation purposes (Hazevoet 1995). This is an arguable point, if for example the recognition of two species of orang utans, or two species of tigers (see above), leads to confusion amongst political decision makers or exasperation with the shifting grounds of argument propounded by conservationists, or if conservation efforts are dissipated by being spread more thinly over more narrowly defined and difficult-to-recognise taxa, smaller populations of which are protected less effectively within more, smaller, scattered reserves. Certainly, scientific positions ought not to be dictated by conservation priorities (Collar 1997).

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