DNA technology and our understanding of the evolution of Australia's avian fauna

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Two features of avian general biology have had a significant influence on our understanding of evolution within the group. First, birds are visual and auditory species and all aspects of their behaviour, including species recognition signals, involve either one or a combination of these senses. Most, if not all, bird species differ in some plumage or song character. Being a visual and auditory species as well, humans can also recognise and identify these avian signals. Consequently, the recognition of avian species is a relatively easy procedure for taxonomists compared to the task in such groups as small mammals which often use scent and pheromones as species markers.

A second feature, flight, has hampered our understanding of higher level systematics in birds. All birds fly or have evolved from flying ancestors. This has placed several constraints on the physiology and morphology of the avian form. Unlike mammals, which are as diverse as whales, bats, elephants and mice, birds require a specialised body form to allow flight. Convergence thus becomes a major problem in unravelling the evolutionary history of birds. Birds which occupy similar niches will resemble one another even though they are unrelated; the independent evolution of aerial feeding in the *Apodiformes* and *Passeriformes* has given rise to remarkable similarities between the unrelated swifts and swallows.

The problems of such convergences combined with a poor fossil record for birds has seen ornithologists become prime movers in adopting molecular techniques for use in systematic studies. These techniques fall into two broad groups: protein based and DNA based.

Protein electrophoresis and microcomplement fixation are two protein based techniques that have been applied with varying success to studies of avian systematics. A general feature of protein evolution in birds is that it proceeds of a rate 3 to 5 times slower than in other vertebrates (Avise and Aquadro 1982). Consequently, protein electrophoresis has been found to be most useful at the generic and familial levels (Christidis *et al*. 1988; Lanyon and Zink 1987; Christidis and Schodde 1991). It is a relatively quick and cost efficient technique which requires around 500 mg of high quality frozen tissues samples of muscle, liver or heart.

Microcomplement fixation is an immunological technique which measures changes in the albumin or transferrin molecule. This is a relatively inefficient technique which requires plasma obtained from 1-2ml of whole blood. Because of the slow rate of albumin and transferrin evolution in birds, microcomplement fixation his rarely been used successfully for determining systematic relationships in birds (Baverstock *et al*. 1991, 1992).

Although protein electrophoresis continues to be a useful tool in avian systematic studies (Christidis *et al*. 1991), attention is now concentrating on DNA based techniques. The earliest and most comprehensive studies were based on DNA-DNA hybridisation (Sibley and Ahlquist 1983, 1985). This technique requires at least 10g of frozen or alcohol preserved tissue.

DNA hybridisation is based upon the double-stranded nature of the DNA molecule in which nucleotides on opposing strands are held together by hydrogen bonds. When double-stranded DNA is heated to 100°C, the hydrogen bonds between complementary base pairs are broken and the opposing strands separate. Subsequent cooling of the solution facilitates reannealing of the complementary stands. DNA from two different species can be combined, denatured, and then allowed to reassociate. The double-stranded molecules that form between complementary strands from the two species will contain base pair mismatch because of their evolutionary divergence. This mismatch means that the heteroduplex (DNA molecule composed of strands from two species) will disassociate at lower temperatures than the homoduplex (single species DNA molecule). This depression of melting temperature serves as an index of divergence between the DNAs under comparison.

The most significant results obtained from DNA-DNA hybridisation studies of Australian birds relate to the relationships of the Australasian songbirds or passerines (Sibley and Ahlquist 1985). When first observed by the early explorers, Australian songbirds were found to share striking similarities with Eurasian families such as the wrens, warblers, robins, babblers and flycatchers. Accordingly, the same European names were applied to Australian genera. Implicit in this taxonomy was the idea that the Australian avifauna was derived from Eurasian stocks. Conventional theory (Mayr 1944; Keast 1981) had it that Australia received its basic stocks of land birds from Asia. They arrived in a sequence of waves across Indonesian island stepping stones over the last 30 million years. The first wave gave rise to now endemic families such as the lyrebirds and honeyeaters while subsequent waves comprised the ancestral wrens, warblers and flycatchers. Arrival was by two pathways, one older via the Lesser Sundas, leading to the development of open forest and drier woodland bird faunas; and the other was newer via New Guinea, leading to a recent ingress of rainforest species down the east coast of Australia.

DNA-DNA hybridisation studies (Sibley and Ahlquist 1985) have demonstrated conclusively that morphological similarities observed between Australian and Eurasian songbirds represent convergences and not shared recent ancestry. The similarities are the result of shared similar life habits such as bark (treecreepers) or ground (wrens) foraging. The results indicate that morphologically diverse groups in Australia such as the honeyeaters (*Meliphagidae*), warblers (*Acanthizidae*) and wrens (*Maluridae*) are part of an adaptive radiation which parallels that of the marsupials. It now appears that these groups evolved within Australasia and as they adapted to new niches, they diverged morphologically so that phylogenetic relationships were obscured. These DNA-DNA hybridisation results have been confirmed by a protein allozyme study (Christidis and Schodde 1991). The allozyme study has provided greater resolution of the links between lineages of Australian songbirds revealing that morphological similarities between robins (*Petroica*, *Eopsaltria* etc) and whistlers (*Pachycephala*) also represent convergences within Australia. The robins are closer to honeyeaters while the whistlers are closer to magpies and crows. Furthermore, a combination of the DNA-DNA hybridisation and allozyme results suggests that the world's songbirds in fact arose and evolved in the southern continents and subsequently spread into Eurasia (Christidis and Schodde 1991) - a complete reversal of conventional theory.

Another indirect method of analysing DNA sequence variation involves the comparison of the number and size of fragments produced by digestion of DNA with restriction endonucleases. Restriction endonucleases are enzymes that cut DNA at a constant position within a specific base pair recognition sequence involving from 4 to 6 base pairs. Either total DNA or purified mitochondrial DNA is isolated, cut with restriction enzymes and run on an electrophoretic gel. The DNA is then visualised with labelled probes that are specific for different types of DNA. Variations in the presence or absence of such sites are termed restriction fragment length polymorphisms (RFLPs).

RLFP analysis is more laborious and expensive than DNA-DNA hybridisation but provides information on the nature, as well as the extent, of differences between DNA sequences. Either frozen or alcohol preserved tissues can be used and the amount required depends on the type of DNA (eg mitochondrial, ribosomal or repeat sequences) being investigated, but usually ranges from 1-5g.

The choice of DNA examined in RFLP studies depends on the level of resolution required. Probes for highly variable repeat sequences in the nuclear DNA ("mini-satellites") are used for genetic "fingerprint" studies. Their major use is in determining parentage and population genetic studies. Few "fingerprint" studies have been conducted on the Australian avifauna. One such study on the Zebra Finch (*Taeniopygia guttata*) by Birkhead *et al*. (1990) revealed significant levels of extra-pair matings and egg dumping in breeding colonies. This appears to be a common feature of colony breeding birds (Morton *et al*. 1990). Such information has important implications for the management of captive populations of rare species when more than one pair are housed together.

At the other extreme, RFLP analysis of ribosomal DNA is useful mainly at the higher level classifications. Mindell and Honeycutt (1989) successfully used RFLP analysis of nuclear ribosomal DNA to examine links between the major clades of birds. No studies directed specifically to the Australian avifauna have been conducted using this approach.

RFLP analysis of mitochondrial DNA is more commonly used in taxonomic studies as it is applicable at population, species and generic levels. Although RFLP analysis of mitochondrial DNA has been used extensively to examine systematic relationships within families of North American birds (Kessler and Avise 1984; Zink *et al*. 1991), few such published studies exist for the Australia avifauna. Studies on relationships within the rosellas (*Platycercus* - Ovenden *et al*. 1987) and Australian babblers, (*Pomatostomus* - Edwards and Wilson, 1990) examined few taxa and were limited in their scope. Because mitochondrial DNA is inherited maternally in a clonal fashion, it is also useful for detecting evidence of hybridisation. Hybridisation and introgression between well differentiated species have been recently detected by RFLP studies in the Australian scrubwrens (*Sericornis* - Joseph and Moritz, 1993) and silvereyes, (*Zosterops* - Degnan and Moritz, 1992). These studies indicate that natural hybridisation between bird species may be more common than previously thought.

Given the utility and resolving power of RFLP analysis, further use of the technique will provide a clearer understanding of the origins and subsequent radiations of Australia's avifauna.

The most precise - albeit expensive and time consuming - method of determining DNA variation between species is direct sequencing of specific genes. The traditional method of doing this is to clone the desired gene into a plasmid, amplify it in bacterial culture and then sequence it. An alternative approach is to amplify the DNA via the Polymerase Chain Reaction (PCR) (Saiki *et al*. 1988). The approach here is to exponentially amplify a segment bounded by specific primers through repeated rounds of DNA replication. Using this technique, large amounts of a specified sequence can be obtained from minute amounts of material. Furthermore, any replication errors which can be introduced through cloning are eliminated. Most studies on birds have used the PCR technique to amplify the mitochondrial

cytochrome b gene for sequencing (Edwards *et al*. 1991; Lanyon 1992). This gene has proven useful for examining questions at species, genera and family levels.

A major advantage of the PCR technique is that it allows the amplification of short (typically < 200 bp) segments from dried museum specimens, fossil, or animal remains (Paabo *et al*. 1989; Thomas *et al*. 1990). Recently, Leeton *et al*. (1992) have been able to amplify the entire cytochrome b gene from single feathers of 100 year old museum specimens including extinct species. One study using the procedure has been aimed at determining the relationships of the recently rediscovered Night Parrot (*Geopsitticus occidentalis*). The lack of skeletal remains on anatomical material of the Night Parrot has hampered our understanding of its relationships. By utilising a combination of museum skins and frozen tissues, Leeton *et al*. (in prep) were able to compare the cytochrome b sequence of several Australasian parrot species including the Night Parrot. Their results revealed a close relationship between the Night Parrot and Ground Parrot (*Pezoporus wallicus*) and aligned the two genera with the *Neophema* parrots. Furthermore, the New Zealand Kakapo (*Strigops habroptilus*) was found to be unrelated to the Night Parrot - similarities in plumage and habits between the two represent convergences. Despite the obvious potential of PCR sequencing, relatively few researchers have applied the technique to studies of the Australian avifauna. Cost of equipment and time appear to be the limiting factors.

Apart from PCR sequencing of DNA, the success of systematic studies using the other molecular techniques described above depends upon the availability of suitable tissue material. Museums and some universities are now collecting and storing tissue samples for future molecular studies. Deficiencies in current tissue banks are of rare Australian species as well as from representative non-Australian genera. Many such species are kept either in zoological parks or in private collections. When these specimens ultimately die, tissue material is rarely collected. Veterinarians can play an important role in further molecular studies by ensuring that such material from rare Australian or exotic species is kept. For most techniques, tissue stored in a household freezer is perfectly acceptable, thus simplifying the procedure. Most state museums, and in particular the Museum of Victoria, are willing to provide the necessary storage tubes, advice, long-term freezer storage etc for such endeavours.

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