



Secrets from the Swamps

Diary extract:

xx August?1983.

"We were camped by the headwaters of a small creek in the jarrah forest near Boranup. Dusk arrived and the spring evening descended. A familiar but unexpected sound '...tk tk tk tk tk ...' came from the dense gully vegetation near the campsite. It was similar to the call of the Karri frog (*Geocrinia rosea*), but they were thought to only occur 70 kilometres to the east in the main karri forest." The only other *Geocrinia* species: the Walpole frog (*Geocrinia lutea*), was known from only nine specimens collected near Walpole in 1963.

Some careful listening and the collecting of a few frogs revealed that these were not the Karri frog: the new frogs were a different colour and had a distinctly different call. We named them White-bellied frogs (*Geocrinia alba*). This find and the later discovery of another previously unknown form of *Geocrinia* in the western Blackwood Plateau, which we named the Orange-bellied frog (*Geocrinia vitellina*), have some important implications for understanding how different species have arisen and the patterns of biological diversity found in the south west.

The subdued topography of the south-west of WA. has long been an enigma to researchers seeking to explain the high species diversity frogs in the region. Without major geographical barriers such as mountain ranges or encroaching seas to separate and isolate populations, researchers have proposed a model of repeated invasions by eastern Australian frogs which ceased with the formation of the Nullarbour Plain, rather than the evolution of new species within the south west itself. However, recent studies of three groups of frogs; *Crinia*, *Heleioporus* and *Neobatrachus* have suggested that trans-Australian migrations may not be the only explanation. Rather, there appears to be mechanisms that would allow in-situ/on-site speciation and our work on the 4 species of *Geocrinia* (the *G.rosea* complex) in the south west provides evidence for one such mechanism.

We set out to examine the distribution and differentiation of members of the *Geocrinia* complex previously thought to occur only in a small area of karri forest in south-west

Australia. The distributions of these species were determined by extensive road transects over much of the south-west. Known occurrence was determined from records of males heard calling during the breeding season.

The Karri frog was found to be widely distributed over the western portion of the main karri belt. It is separated from the Walpole frog by a distance of about 40 km corresponding to the Pingerup Plains, north of Broke Inlet. The Walpole frog was found only at sites within a 12 km radius of Walpole. Both species are common within their ranges.

Because of their breeding biology, members of this species complex are commonly associated with creek systems, particularly those having soil with a high organic content that do not readily dry out over summer. However, at the centre of the range of the Karri frog, in the areas where karri occurs over the whole landscape, males may be heard calling from rotting logs well away from creek systems. All streams and sites low in the landscape profile include suitable habitat for this species.

Having relatively restricted in distribution from a conservation viewpoint, both the Karri and Walpole frogs are thought to be secure as a large proportion of their current distributions lie on publicly owned land. Although initially reduced, their populations persist through logging and burning are again abundant within 2-3 years.

The comparatively dry Blackwood Plateau separates the Karri and Walpole frogs from two species found to the north-west of the Blackwood River. Both are very restricted and have smaller populations in an area of drier, more seasonal climate than that found where the Karri and Walpole frogs occur. Both species have been gazetted as threatened fauna, the only frogs in Western Australia to have this dubious honour.

The White-bellied frog (*G. alba*) occurs over an area of about 100 square kilometres however suitable breeding sites are restricted to 1.9 square kilometres within the Witchcliffe, Karridale area chiefly on privately owned land. It is found in the only corridor of forest remaining that

remains uncleared, much is privately owned and therefore potentially subject to clearing. Clearing for agriculture and subsequent grazing destroys the environment necessary for members of this species complex. The protection of sections of creek systems suitable for this species may be sufficient to ensure its survival in areas of agricultural land.

The Yellow-bellied frog (*G. vitellina*) has a range of only six square kilometres and within this is confined to a small area (0.2 km²) of unusual habitat in five creek systems in State Forest to the north of the Blackwood River. This is probably the most restricted of any frog found in Australia. Obviously, there are considerable hazards associated with having such a small range.

But what is a species?

Is it important that we differentiate species or could we consider all the 4 frogs to be variants of the Karri frog?

The biological species concept defines species as groups of interbreeding populations reproductively isolated from other such groups. Reproductive isolation might be caused by behavioural barriers. For example, in frogs, males call to attract mates. If there is a change in structure of the call females may simply not recognise the new call as that of a male therefore not mate with them. Alternatively, different species may mate, but be genetically too different to produce viable offspring.

Although the biological species concept is a popular one, in reality the problem of determining whether these four frogs could successfully interbreed when each of their populations is isolated is a difficult one. For similar but geographically separated forms, the best we can do is assess whether the differences between them would act as barriers to reproduction if they ever came into contact.

Hence, we investigated differences in male call, in colour and morphology and levels of genetic differentiation using electrophoresis. Colour and pattern differences between the four frogs are shown on the **distribution map**. All four are readily distinguishable by colour. We measured 11 aspects associated with size and shape of the head, body and limbs but were unable to find any consistent differences between them.

The calls of the Karri and Walpole frogs are very similar. Both frogs produce a 'tk' sound more or less continuously. However, the two frogs found further west produce a distinct call composed of 12-15 'tk' sounds. The calling seasons of all types are similar: spring to mid- summer.

Genetic differentiation was assessed by electrophoresis. Electrophoresis separates organic molecules suspended in a starch gel. Molecules move in response to an electric field applied directly to the gel. Using 12 different enzymes and proteins collected from frog samples we measured the variation in distance the organic molecules moved along the starch gel. From this we produced an index of Genetic Distance: a measure of the genetic difference between the frogs.

There are very few genetic differences between populations. However, each of the frogs are clearly distinct. The Genetic Distance between the Karri and Walpole frogs is comparable with that between *Crinia insignifera* and *C. pseudinsignifera*, two species found along the Darling Scarp which are known to interbreed. The Genetic Distance between the Karri frog and the Orange-bellied frog is much greater and is comparable with that between *Crinia glauerti* and *C. georgiana*, two frogs found together throughout the south-west but do not interbreed. While the Genetic Distance index gives us some indication as to whether the four frogs are likely to be separate species, it would be unwise to use a single measure to decide species status.

If the range of the Karri and Walpole frogs came into contact it is difficult to predict what would happen. It seems likely that they would interbreed as there are few behavioural differences. Similarly the Orange-bellied and White-bellied frogs would probably interbreed, but there is little chance that the Orange-bellied or White-bellied frogs would interbreed with the Karri frog as there are major differences in calls and significant genetic differentiation. These differences are regarded as sufficient to enable us to conclude that each of the four frogs are separate species.

Irrespective of their taxonomic status, however, conservation programs should emphasize the important differences between these four sets of isolated populations.

The Walpole frog is separated from the Karri frog by 39 kilometres of swampy land that dries in summer. The Karri frog is separated from the Orange-bellied frog by about 47 km of rolling terrain including laterite uplands, swamps and narrow stream channels. The Orange-bellied and White-bellied frogs are separated by 9 km's of laterite uplands and narrow valleys. These factors which are thought to be currently maintaining the gaps in the distribution of species may be different from those responsible for originally separating the species. However they do serve to demonstrate that

even in areas with relatively small variation in topography, geographic barriers of a few kilometres can be maintained.

Hence despite the subdued topography of the south-west, it is possible to isolate frog populations and for them to subsequently evolve into new species. This is in dramatic contrast with views expressed in the 1950's.

When did these species become isolated?

One approach to this question would be to look at the climatic and geological history of this area and find a set of events that might have produced splits in a continuous range to give the species. The difficulty is that there might be several sets of likely events and there may be no logical way to choose between them. Clearly the current distribution pattern of species in the south-west suggest a complex history of climate change.

Genetic Distance can offer some clues as to when these events might have occurred. One of the more surprising results that has come from studies of molecular evolution is the discovery that molecular structure seems to evolve at a relatively constant rate over time spans of millions of years. For amphibians, a Genetic Distance value of 1 equates with a divergence date of about 18 million years. Applying this to the four frogs, we estimate that the Karri and Walpole frogs were split about 5 million years ago, and that the Orange-bellied frog split from the Karri frog about 15.5 million years ago.

Because most of the larger and more mobile vertebrates such as birds and mammals are widely distributed in the south-west, their distribution patterns are of limited value in providing insight into past environments, particularly in areas of limited topographic change. Current research on crustaceans, spiders and on small vertebrates such as cryptic lizards suggests that this research on *Geocrinia* is but the tip of the iceberg in understanding the complex patterns of species distributions in the south-west.

