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Aridification drove repeated episodes of diversification between Australian biomes: Evidence from a multi-locus phylogeny of Australian toadlets (*Uperoleia*: Myobatrachidae)

Renee A. Catullo [a,b,*], J. Scott Keogh [a]

[a] Evolution, Ecology & Genetics, Research School of Biology, The Australian National University, Canberra, Australia
[b] CSIRO Land & Water, GPO Box 1700, Canberra, ACT 2601, Australia

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ABSTRACT

Australia is a large and complex landmass that comprises diverse biomes ranging from tropical rainforests to harsh deserts. While Australian biotic diversity has evolved in response to landscape and climate changes, evidence of Miocene or later biome shifts are few. The Australo-Papuan endemic frog genus *Uperoleia* is widely distributed across mesic, monsoonal tropic and arid regions of Australia. Thus, it represents an ideal system to evaluate biome shifts as they relate to known landscape and climate history. We comprehensively sampled the distributional range of 25 described *Uperoleia* species and generated a detailed molecular phylogeny for the genus based on one mitochondrial and five nuclear loci. Our results support a single origin of monsoonal tropic taxa, followed by diversification within the region under the influence of the Australian monsoon. Molecular dating analyses suggest the major divergence between eastern mesic and monsoonal species occurred in the Miocene approximately 17 million years ago, with repeated evolution of species from monsoonal biomes to arid or mesic biomes in the later Miocene, early Pliocene and at the beginning of the Pleistocene. Our detailed sampling helps to clarify the true distributions of species and contributes to on-going work to improve the taxonomy of the genus. Topological differences between nuclear and mitochondrial phylogenies within major clades suggest a history of mitochondrial introgression and capture, and reduce the ability to resolve close interspecific relationships.

1. Introduction

Biomes are communities of flora and fauna occupying a major habitat defined by broadly similar climate or landscape. Although terrestrial species are usually confined to one particular biome, the distribution of closely related species across biomes can reveal patterns of speciation as they relate to biome history (Coyne and Orr, 2004). Within biomes, phylogeographic studies have shown that past climatic fluctuations have driven diversification. In the Northern Hemisphere, Pleistocene glaciation drove lineages to refugia, where they subsequently diverged through allopatry and drift, or through adaptation to available environmental niches (Hewitt, 2004). In Australia, although there was little glaciation, extreme aridification linked to Pleistocene cycles also drove lineages into refugia (Catullo et al., 2013; Garrick et al., 2012; Pepper et al., 2011a), with similar patterns of subsequent diversification. Australia also developed extremely xeric biomes into which many species diversified, often during the less xeric interglacial periods (Chapple et al., 2011; Rabosky et al., 2007).

Modern-day Australia is divided into three major biome types – the monsoonal tropics, the arid zone, and the winter-rainfall mesic regions in the southwest and east (Fig. 1). The monsoonal region of Northern Australia (Fig. 1, green) is defined by having a summer wet season associated with cyclonic rainfall, and a dry winter season (Bowman et al., 2010). Although the monsoonal tropics have been defined as mesic in terms of total rainfall (Fujita et al., 2010), this biome differs substantially from other Australian mesic regions, which have winter rainfall. Australia has two independent winter-rainfall mesic regions (Fig. 1, purple & light gray). The eastern mesic zone has been defined in various ways; we follow Byrne et al. (2008) and Fujita et al. (2010) in including the semi-arid Murray–Darling basin, which receives substantially more rainfall than does the arid zone (Smith and Chandler, 2010). The south-western mesic zone is separated from the eastern mesic zone by the arid

* Corresponding author. Current address: CSIRO Land & Water, GPO Box 1700, Canberra, ACT 2601, Australia.
E-mail address: renee.catullo@anu.edu.au (R.A. Catullo).

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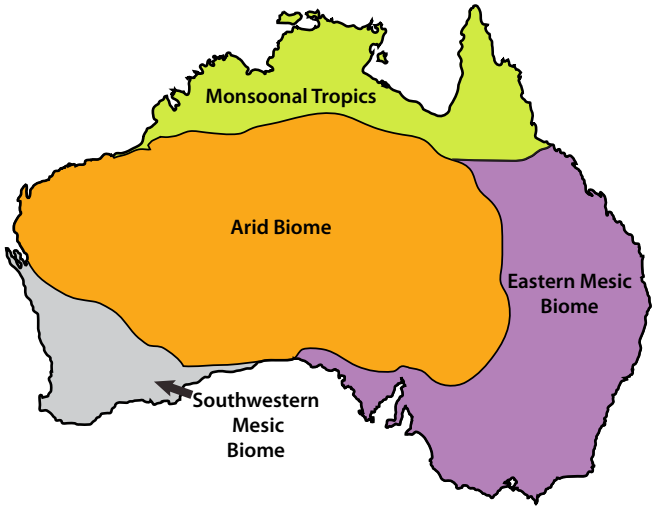

Fig. 1. Major biomes of the Australian mainland (colors). Modified from Byrne et al. (2008), Catullo et al. (2013), Chapple et al. (2011), Fujita et al. (2010), Potter et al. (2012).

deserts of Australia. The arid zone (Fig. 1, orange) is a massive xeric region occupying approximately 70% of the Australian landmass, and is characterized by intense aridity and highly variable rainfall (Byrne et al., 2008).

Recent reviews of historical biogeography within each of the biomes have been completed. Patterns of diversification within the arid zone have been extensively reviewed in Byrne et al. (2008) and Pepper et al. (2011a,b), with these studies demonstrating that mountains likely acted as refugia during hyper-arid climate cycles, playing a role similar to non-glaciated regions of the Northern Hemisphere in the generation of diversity. A general overview of diversification in mesic regions of Australia is provided in Byrne et al. (2011), while repeated patterns of diversification in the eastern mesic zone are reviewed in Chapple et al. (2011). The first reviews of broad-scale biogeographic structure in the Monsoonal Tropics were on *Heteronotia* geckos (Fujita et al., 2010), saxicolous rock wallabies (Potter et al., 2012) and alluvial *Uperoleia* frogs (Catullo et al., 2013). However, well-sampled studies of the patterns of diversification between biomes are rare.

Various lines of evidence suggest that Australian mesic environments are the ancestral environments for most Australian biota (reviewed in Byrne et al., 2011; Chapple et al., 2011). In the last 20 million years, Asian-origin taxa became a significant component of the monsoonal tropics region, likely through the increasing proximity of the Australian continent (reviewed in Bowman et al., 2010; Crisp and Cook, 2013). Studies of diversification of arid zone taxa have supported two patterns: a single, old origin of arid taxa followed by diversification, or recent and repeated evolution of arid tolerance (reviewed in Byrne et al., 2008). Fujita et al. (2010) reported multiple transitions between monsoonal and arid lineages in the gecko *Heteronotia binoei*, although lineages were not always found exclusively in a single biome. This study reconstructed a monsoonal origin for arid lineages, with transitions linked to cycles of aridification in the Pliocene and Pleistocene. *Heteronotia* entered Australia from Asia in the Miocene and does not occupy the mesic regions of Australia; thus, this study was unable to address the questions of relationships between all major Australian biomes, and was limited to the last 5 million years.

In this study we examine patterns of between-biome diversification in the frog genus *Uperoleia* (family Myobatrachidae). The Myobatrachidae has an ancient evolutionary history associated with the Australo-Papuan landmass that far pre-dates the current proximity of the Asian continent (Frost et al., 2006; Read et al., 2001). Biota that have existed on the continent since Gondwanan times are primarily distributed in the eastern or southwestern mesic regions (Crisp and Cook, 2013; Slatyer et al., 2007). However, most diversity in *Uperoleia* exists in the northern monsoonal tropics region of Australia, with a significant number of species in both the arid zone and eastern mesic region. Thus, *Uperoleia* species are ideally suited to examine of the role of climate and landscape on diversification in Gondwanan biota.

We use dense taxon sampling for all available species of *Uperoleia* and a large multi-locus data set to generate a robust molecular phylogeny for the genus based on multiple independent loci. We then use this phylogeny, and the timing of divergence events, to evaluate patterns of diversification between the three biomes inhabited by *Uperoleia*. Our specific goals were to (1) establish the evolutionary relationships between *Uperoleia* species; (2) identify the biome of origin of the *Uperoleia* genus; (3) examine the influence of climatic fluctuations on the evolution of arid distributed *Uperoleia*; and (4) determine whether tolerance of aridity evolved once or multiple times. In addition, our fine-scale sampling allows us to quantify phylogeographic structure within 25 of the 28 recognized *Uperoleia* species and to address problematic issues relating to taxonomy and species distributions.

2. Materials and methods

2.1. Field collection of specimens and tissue samples

We addressed three issues when choosing our specimens for genetic analysis and targeting additional fieldwork: (1) maximum geographic spread for each species; (2) possible contact zones between parapatric species; and (3) geographical gaps in existing collections. In total, we used 589 tissue samples representing 25 of the 28 described *Uperoleia* species. The three species not represented were *U. marmorata* (not seen since 1841), *U. orientalis* (not seen since the early 1900s, (Tyler et al., 1981a)), and *U. arenicola* (not collected since 1978, and we were unsuccessful in recent field trips to find it). *Spicospina flammocaerulea* was used as the outgroup in all analyses as it represents the monotypic sister genus to *Uperoleia* (Frost et al., 2006; Read et al., 2001). Specimen data are provided in Appendix 1, and distribution maps of tissues are illustrated in Fig. 2. Data points in Fig. 2 represent the individuals in the mitochondrial phylogeny, except where the specimen carries a different nuclear DNA haplotype, as discussed below. We were unable to acquire or map explicit location details for museum tissues numbered Up0816-824 (*U. aspera* & *U. mjobergi*), or Up765-770 (*U. inundata*) but included the tissues in our genetic analyses to compensate for the low total number of available samples, especially for *U. aspera* and *U. mjobergi*.

2.2. DNA extraction, amplification, and sequencing

For all 589 samples, including the outgroup species *S. flammocaerulea*, we generated a 783 base pair (bp) *16S* data set to obtain an estimate of the number of species lineages in the genus. We selected 294 of the samples as representatives of every *16S* clade for additional sequencing for five nuclear exons (Table 1). Of the 589 samples included in this study, 342 individuals have not been included in any previous studies of the genus.

Genomic DNA was isolated using Proteinase K digestion and a modified sodium acetate extraction. Table 1 lists the oligonucleotide primer pairs used in PCR amplification and sequencing, as well as PCR amplification protocols and gene length. PCR products were purified using EXOSAP-IT (Affymetrix, Inc.). BIGDYE TERMINATOR 3.1 (Applied Biosystems) was used for cycle sequencing, and capillary electrophoresis was completed on an ABI 3130XL GENETIC ANALYZER