

Desert spring refugia: museums of diversity or evolutionary cradles?

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Refugia play a critical role in preserving species unable to move or adapt to cope with environmental change. The role of refugia as ‘museums of diversity’ means these environments have a high conservation priority. Less well known, however, is the role that isolated and fragmented refugia can play in the generation of new diversity. Here, we examined the diversification and evolutionary history of a community of endemic invertebrates that inhabit Australian desert spring refugia. We compared the phylogenies of seven endemic groups (*Haloniscus* and *Phreatomerus* isopods, chiltoniid amphipods, *Ngarawa* ostracods, *Trochidrobia* and *Fonscochlea* snails and *Gymnochthebius* beetles) from these springs and examine the rates and timing of diversification and reconstructed the phylogeographic history of each taxon. Despite major life-history differences among these taxa, they demonstrate remarkable similarities in their evolutionary histories. All groups have multiple lineages that extend back to a time before the formation of present-day deserts, and significant geographic-based diversification since adapting to a refugial habitat. The results provide further evidence that refugia act as museums of biodiversity, preserving lineages that would have otherwise gone extinct. However, we also observed that isolation in refugia corresponds with significant diversification, leading to a recently evolved, novel endemic fauna, supporting the idea that fragmented refugia provide ideal conditions for the generation of future biodiversity hotspots.

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Introduction

Refugial habitats act as climatic buffers allowing species to persist and preserving evolutionary history that would otherwise be lost during large-scale climatic change (Kepel *et al.* 2012). As such, these habitats are often considered to be ‘museums of diversity’ as their isolation and climatic stability leads to the preservation of old endemic relicts. Due to this role in preservation, identifying and protecting refugia is a considered to be a major conservation priority (Shoo *et al.* 2013). However, given enough time, and despite an initial loss of diversity (Fig. 1), the fragmented nature of refugia can also lead to the generation of

evolutionary lineages (Wiens & Donoghue 2004). Long established refugia should therefore represent a blend of very old and new lineages (i.e. acting both as museums and cradles of diversity) and provide a unique insight into past and present evolutionary processes. By studying species presently restricted to these fragmented habitats, we can gain an understanding of the dual role that refugial habitats play in both preserving and creating species diversity.

Desert springs are recognized as excellent examples of refugial aquatic habitats (Shepard 1993; Ponder 2002; Kodric-Brown & Brown 2007; Davis *et al.* 2013). In most present-day deserts, ecosystems have changed from a mesic,

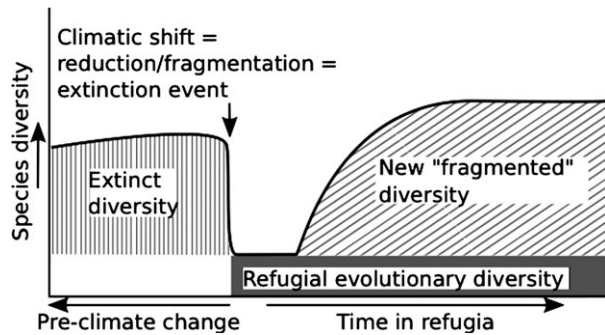


Fig. 1 Conceptual model demonstrating (i) the initial loss of diversity (vertical lines) caused by a major climatic change, which leads to the constriction and fragmentation of suitable habitat; (ii) the preserved diversity (shaded area), which is able to survive in evolutionary refugium; and (iii) new diversity (diagonal lines), which evolves due to the impact of habitat isolation and fragmentation.

system of perennial freshwater streams and wetlands to highly variable and often saline ephemeral desert waterways. To avoid extinction yet remain *in situ*, aquatic organisms must have either adapted to a complete environmental change or have eked out an existence in refuges such as permanent freshwater springs (Johnson 2002; Murphy *et al.* 2009). Globally, desert springs contain numerous endemic taxa, often with parallel life-history characteristics such as poor dispersal ability and low tolerance to desiccation (e.g. Liu & Hershler 2009; Murphy *et al.* 2012). Desert springs are also among the most naturally fragmented ecosystems, making species that rely on them ideal candidates for understanding the evolutionary impact of changing climates and a refugial existence.

Our study is focused on the Lake Eyre desert springs fed by the Great Artesian Basin in central Australia (GAB springs). A unique system of springs that are geographically fragmented throughout the central Australian desert comprises a rare aquatic ecosystem of endemic animals and plants. A significant climatic shift in the late Miocene led to the aridification of inland Australia which has led to arid zone specialization in many terrestrial and freshwater species (Byrne *et al.* 2008), and the restriction of many taxa into fragmented refugial habitats (Leys *et al.* 2003; Guzik *et al.* 2012), including a suite of endemic aquatic invertebrates in the GAB springs (Ponder *et al.* 1995; Murphy

et al. 2009, 2012, 2013; Guzik *et al.* 2012). We hypothesize that the GAB spring community of endemic invertebrates comprises an 'evolutionary cohort' (Carstens & Richards 2007), responding as a whole to environmental change through time. Using this cohort, we aimed to determine the role that the refugial desert spring habitat has contributed to the present-day diversity of the spring community, either by acting as *museums of diversity* and preserving lineages that evolved in a now extinct ecosystem (i.e. prior to aridification of central Australia) or as *evolutionary cradles*, promoting diversification by virtue of the isolated and fragmented nature of spring refuges. Here, we present a comparative analysis of the phylogenetic history, biogeography and diversification of all currently described (*Phreatomerus* isopods, some chiltoniid amphipods, *Ngarawa* ostracods, *Trochidrodia* and *Fonscochlea* snails and *Gymnochthebius* beetles), and some undescribed (*Haloniscus* isopods and some chiltoniid amphipods) endemic aquatic invertebrates across the Lake Eyre GAB springs.

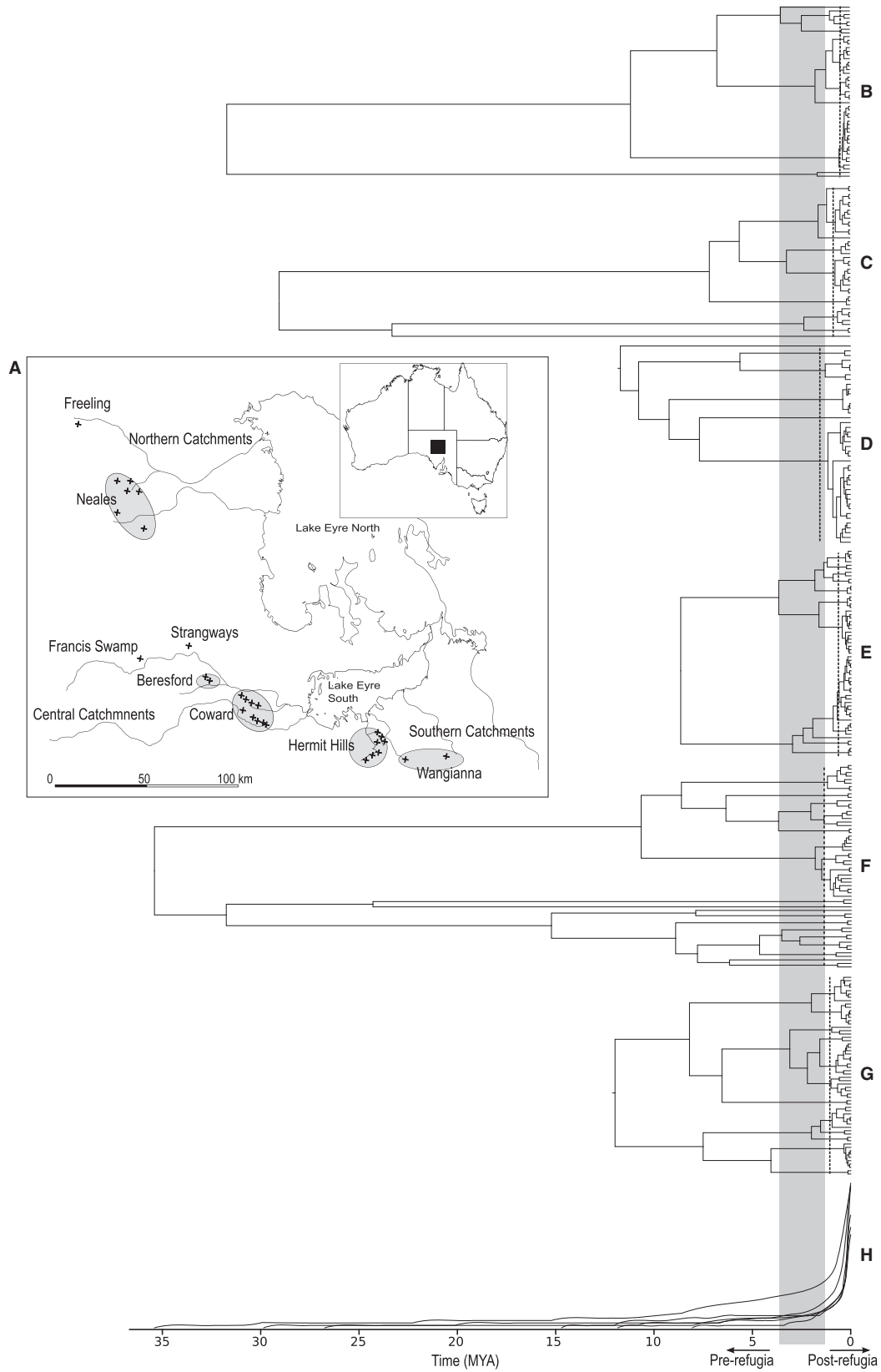
To date, most studies of refugial habitat have concentrated on their role in preserving single groups of species. This system of desert springs and narrow range endemics provides a unique opportunity to examine the role that isolation within refugia has played on the diversification of an entire ecosystem. We aim to shift the focus on refugia from habitats that preserve evolutionary history – to those that actively promote the formation of new evolutionary entities.

Methods

GAB springs

The study focused on the GAB springs situated west of Lake Eyre in central Australia (Fig. 2), where around 3000 springs are located around geological faults over approximately 300 km of desert. The springs are permanent freshwater systems, providing the outlet of the Great Artesian Basin, an underground body of water that stretches across one-third of the continent. Aridification in the Lake Eyre region began in the Miocene and from the late Pliocene, the permanent rivers and lakes disappeared, salt lakes developed and deserts formed 2–4 MYA (Alley 1998; Fujioka *et al.* 2009). It is thought that the springs have an early Pleistocene maximum origin between 1 and 2 MYA and, although difficult to date, the oldest flowing spring

Fig. 2 (A) Map of GAB springs showing location within Australia (inset), the spring complexes, and location of groups of springs (crosses) and the northern, central and southern catchments flowing into Lake Eyre. (B–G) Phylogenetic analyses for the 6 endemic taxa included in this study (B–*Ngarawa* ostracods, C–*Haloniscus* isopods, D–*Gymnochthebius* beetle, E–*Phreatomerus* isopods, F–Chiltoniidae amphipods, G–Hydrobiidae snails). The vertical dotted line on each tree represents the GMYC 'cut-off' between intra- and interspecific divergence. 2H. Lineage-through-time (LTT) plot for each of the taxa. Trees and LTT plot all scaled to the time axis (in millions of years), and the shaded band represents the 'grey zone' where desert and spring formation overlap.



group is around 750 000 years (Prescott & Habermehl 2008). For the purposes of this study, we can group these time periods for the origins of spring endemics as clearly before refugia (>4 MYA), clearly after spring isolation (<1 MYA), and a 'grey zone' (4–1 MYA years) where the formation of deserts and the springs may overlap (Murphy *et al.* 2012).

Spring endemics

DNA sequence data were generated for several endemic groups across the range of the GAB springs. This included the ostracod genus *Ngarawa*, undescribed *Haloniscus* isopods (which are not fully aquatic, but inhabit the wetted edge of a spring), the beetle genus *Gymnochthebius* and the endemic hydrobiid snail genus *Fonscochlea*. These data were combined with DNA sequences from previous phylogenetic/phylogeographic research for the major lineages of two endemic crustacean groups [chiltoniid amphipods (Murphy *et al.* 2009, 2013), and *Pbreatomerus* isopods (Guzik *et al.* 2012)] and an endemic hydrobiid snail [*Trochidobia* (Murphy *et al.* 2012)] (which was analysed concurrently with *Trochidobia*, as an overall Hydrobiidae phylogeny). The present-day distribution of these taxa can be categorized spatially (Fig. 2A, Table S1) based on either the GAB spring complexes (geographically adjacent springs, generally flowing from common geological faults), or their the location in the northern, central or southern ephemeral drainage flowing into the saline Lake Eyre. Taxa were collected across as many spring complexes as possible to ensure a comparative data set, with the three Lake Eyre catchments represented by all taxa (Table S1).

Molecular methods

DNA extraction, PCR amplification of the mitochondrial (mtDNA) *cytochrome oxidase subunit 1* (*COI*) gene region and sequencing of *Ngarawa* ostracods followed Murphy *et al.* (2013), *Haloniscus* isopods and *Gymnochthebius* beetles followed the protocols of Guzik *et al.* (2012), while *Fonscochlea* snails followed the protocol of Murphy *et al.* (2012). Sequence data were compiled using Geneious 5.3 (Drummond 2010), and new sequences submitted to GenBank (KT23598–KT236082). Although there are issues with relying on single gene trees to illustrate phylogenetic relationships, our study is predominantly asking question regarding the timing of diversification events, rather than the reconstruction of common ancestry, and, as demonstrated by Burbrink & Pyron (2011), gene trees provide an accurate estimation of diversification rates. In addition, as opposed to gene tree/species tree concordance to enable confidence in phylogenetic reconstruction, our study uses comparative phylogenetic histories across very divergent taxa where congruence in the diversification of lineages due

to random chance (as opposed to a shared evolutionary history) is highly unlikely.

Phylogenetic, molecular clock and ancestral state estimation

Phylogenetic analyses were undertaken using BEAST version 1.5.2. (Drummond & Rambaut 2007). Models of evolution were as published or determined using MrModeltest (see Supporting Information). A UPGMA starting tree was estimated under the appropriate model, and an uncorrelated lognormal clock was used. The standard arthropod mtDNA molecular clock of 2.3% divergence per million years (Brower 1994) was used to calibrate the clock; however, given this is not taxon specific, a normal distribution prior with a mean rate of $0.0115 \pm 10\%$ standard deviation was used (Murphy *et al.* 2012). Using this method means that the rates used to calibrate clock encompass other reported calibrations such as the 2.5% proposed for subterranean isopods (Ketmaier *et al.* 2003). The exception to this was the hydrobiid snails, which used a taxon specific approach of 0.0176 ± 0.007 per Myr (Wilke *et al.* 2009). Three coalescent models (exponential growth, expansion growth and constant size) and the Yule model for tree priors were estimated and compared. Analyses were run five times for 10 million generations with a burn in of 25% of the total saved trees. Convergence in analyses was assessed using Tracer (Rambaut & Drummond 2007), ensuring effective sample sizes for each analyses were >300).

Ancestral state reconstructions were conducted in BEAST, simultaneously with the phylogenetic reconstruction and molecular clock dating, as specified above. Geographic localities were coded as character states based on either spring complex or Lake Eyre drainage (two separate analyses undertaken for each species). Following Lemey *et al.* (2009), historical dispersal between populations is estimated along the branches of the phylogeny using a continuous-time Markov chain, estimating discrete states as a continuous function of time. Due to the uncertainty and likely complexity of potential connectivity among sites, flat rate priors were assigned to movement between regions.

Diversification

To examine patterns of intraspecific diversification among the species, we used the general mixed Yule-coalescent (GMYC) model, which examines changes from interspecific to intraspecific in branching rates (Pons *et al.* 2006). The time-calibrated trees generated above were analysed using a log-likelihood ratio test to assess the significance of the estimated shift in branching rates (i.e. how well they fit the GMYC model). Distinct entities were delineated based on the lineages that are split by interspecific branching rates. These tests were implemented in the SPLITS package and

both single and multiple thresholds (available from <http://r-forge.r-project.org/projects/splits>) in the R statistical environment (R Development Core Team, 2011).

We acknowledge that GMYC analyses are not ideal for delineating species; however, this was not our aim when using this procedure. Rather, we used the results of the GMYC analyses to provide an estimation within each tree of the timing of the transition from a Yule evolutionary model (which can be best depicted using a bifurcating phylogenetic tree) to a coalescent model (where phylogenetic trees may not depict accurate relationships). With this in mind, we selected the most conservative estimate of lineages (in each case this was the single threshold method in SPLITS). This enabled us to determine where in the trees we could reliably analyse the diversification within each lineage using phylogenetic methods. Using the phylogenetic trees, the tempo of diversification was analysed in R with the packages APE (Paradis *et al.* 2004) and Laser (Rabosky 2006). Lineage-through-time (LTT) plots and γ -statistics were calculated for each taxon. Because incomplete taxon sampling might influence γ -statistics, we conducted a Monte Carlo constant rates (mccr) test with 10 000 replicates, assuming 10% missing species. 10% missing taxa was chosen based on the fact that over all of our research in this region, we sampled approximately 90% of the distinct spring groups – and all taxa studied so far demonstrate similar geographic patterns of diversification; therefore, it is estimated that missing taxa within the groups are likely to be equivalent. We also compared a constant diversification rate (RC-models) with variable diversification rate models (RV-models) using AIC and likelihood ratio tests computed under the program R.

Results

A total of 139 samples from four GAB spring endemic groups were sequenced and, with the 153 selected from previous studies, amounted to a total of 292 samples collected from the 6 groups over the 10 Lake Eyre spring complexes. Detailed phylogenetic results for each group are presented in the supplementary material (Fig. S1); however, robust and well-supported trees were generated for each species, with the great majority of major nodes receiving 0.95 support or greater. The exception to this is *Phreatomerus*; however, the relationships generated in this analysis match exactly those from Guzik *et al.* (2012) (using combined DNA sequences and allozymes); therefore, we are confident in the phylogenetic trees used for further analyses.

Significant evolutionary divergence is found in all GAB spring endemics, with all having multiple lineages that evolved before the formation of the central Australian deserts (~4 MYA) (Figs 2B–G, S1, Table 1). There is some

Table 1 Number of GMYC identified ‘species’, the oldest divergence date (MYA) and the date of coalescence for monophyletic clades from Neales, Coward and Hermit Hills spring complexes. For all dating, the 95% confidence intervals are shown

	GMYC	Oldest divergence	Neales	Coward	Hermit Hills
<i>Gymnochthebius</i>	6 (4–20)	4.6–37.4	NA	0.31–3.60	0.19–0.59
Chiltoniidae	19 (18–28)	23.9–47.2	3.11–6.16	5.82–11.33	1.19–2.49
Hydrobiidae	12 (8–17)	8.2–16.2	0.90–2.60	0.43–1.30	0.08–0.50
<i>Ngarawa</i>	18 (14–26)	18.9–47.4	2.03–4.40	NA	NA
<i>Haloniscus</i>	9 (7–14)	18.7–40.8	1.5–3.4	0.73–1.77	0.29–0.89
<i>Phreatomerus</i>	16 (12–20)	4.8–13.5	1.0–2.8	0.51–1.46	0.21–0.78

variation around the age of the oldest divergence event for each taxon (Table 1), with the oldest (ranging from 18–47 MYA) found in amphipods, ostracods and *Haloniscus* isopods (all showing considerable overlap), while the youngest are the hydrobiids and *Phreatomerus* (ranging from 4.8–16.2 MYA) (which also overlap considerably with each other). Remarkably, across all taxa, GMYC analyses suggest that the boundary between intra- and interspecific divergence occurs at the more recent boundary of the ‘grey area’ (Fig. 2B–G), around 1–2 MYA. The results of the GMYC analyses suggest that the Yule model is appropriate for the majority of evolutionary time covered by each phylogenetic tree; therefore, lineage-based diversification analyses are appropriate.

The lineage-through-time analyses of all taxa resulted in remarkably similar plots (Figs 2H, S2), showing a low, steady diversification rate and an exponential increase in the last 1 million years (albeit the results of the GMYC analyses suggest that this latter increase represents intraspecific diversification). Accordingly, the γ -stat results (Table S2) were similar across taxa, with none showing a significant result, suggesting there has been no slowdown in diversification. For all taxa, AIC analyses rejected multirate models of diversification, and the birth–death model of evolution was supported (Table S2).

To aid comparison among the taxa, divergence events were scaled based on the possible number of divergence events in each tree ($n-1$) (Table 2). There was reasonable variation among the taxa for the proportion of divergence events that occurred in the oldest time period (>4 MYA), ranging from 0.26 in chiltoniid amphipods to 0.05 in phreatoicid isopods. Likewise, there is considerable variation across taxa in divergence events occurring in the most recent period (<1 MYA – after spring formation), ranging from 0.24 in the beetles to 0.89 in the ostracods, but this needs to be interpreted cautiously as these results are based on coalescent-based branching processes. In contrast, the proportion of divergence events in the 1–4 MYA (grey area) range is quite similar across all taxa (Table 2).

Table 2 Proportion of total divergence events (scaled against total possible divergence events – $n-1$) for each time category, proportion of divergence events between lineages from different surface catchments and proportion of divergence events between different spring complexes

	Proportion of total divergence events			Divergence between different surface catchments			Divergence between spring complexes	
	>4	1–4	<1	>4	1–4	<1	1–4	<1
<i>Gymnochthebius</i>	0.12	0.26	0.24	0.60	0.36	0.10	0.55	0.30
Chiltoniidae	0.26	0.21	0.72	0.40	0.00	0.00	0.08	0.00
Hydrobiidae	0.08	0.18	0.77	0.40	0.45	0.04	0.64	0.24
Ngarawa	0.06	0.17	0.89	0.22	0.13	0.02	0.38	0.14
<i>Haloniscus</i>	0.10	0.15	0.51	0.50	0.17	0.00	0.50	0.00
<i>Phreatomerus</i>	0.05	0.15	0.88	0.67	0.22	0.00	0.44	0.08

Very similar patterns of localized diversification were evident across all taxa, particularly in the most recent time period. Most taxa showed large monophyletic clades for each of the three largest spring complexes (Neales, Coward and Hermit Hills). More significantly, with the exception of the amphipods, all other taxa show overlapping maximum ages of divergence in these three spring complexes (Table 1), with the Neales lineages the oldest, while the Hermit Hills lineages were generally the youngest. The proportion of divergence events that led to an ancestral switch between spring complexes (Table 2) in the last 1 million years ranged from 0 in amphipods and *Haloniscus* isopods to 0.3 in the beetles. Within the 1–4 MYA time period, the proportion of divergence events between spring complexes was much higher and, with the exception of amphipods, quite similar among taxa (0.4–0.6).

Ancestral state reconstruction based on river basins (Fig. S3) showed divergence events that involve lineages splitting between surface catchments. All taxa, with the exception of the *Haloniscus* isopods have ancestral origins in all three drainages prior to the formation of deserts >4 MYA. In addition, we found that all taxa showed multiple origins for the northern drainage, with many also having multiple origins in the southern and central drainages. Within the last 1 million years, these divergence events between drainages are zero, or very low (<0.05), with the exception of the beetles, where around 10% of divergence events involve divergence across drainages. Interdrainage divergence events were higher in the 1–4 MYA than the <1 MYA time period and, in general, lower than suggested for >4 MYA, where roughly 50% of divergence events can be attributed to lineages moving between drainage basins.

Examining the four new data sets in detail (*Ngarawa* ostracods, *Haloniscus* isopods, *Gymnochthebius* beetles and

Fonscochlea snails) (Fig. S1), like previously studied GAB spring taxa, there is evidence of previously unrecognized species-level diversity. This is particularly evident within Ngarwa, which presently consists of a single described species (*N. dirga*) from all spring groups and complexes. Clearly, the phylogenetic results demonstrated in Fig. S1, and Table 1 demonstrate that this group consists of multiple lineages (at least four that evolved prior to the formation of deserts (including up to 30 MYA) and many more that demonstrate the geographic isolation evident in species of amphipods (Murphy *et al.* 2013) and snails (Ponder *et al.* 1995). The same patterns are evident within the presently undescribed *Haloniscus* isopods, which clearly also do not consist of a single widely distributed species. Clearly, in each of these groups, there is a need for further molecular and morphological studies to identify the true species richness in this environment.

Discussion

There is a remarkable congruence among the evolutionary patterns of diversification for the GAB spring taxa, suggesting that these species do represent an evolutionary cohort. We observed matching patterns across taxa in: (i) diversification through time of all taxa shows an exponential increase in lineages and intraspecific boundaries forming at a similar point in time (as identified by GMYC); (ii) ancestral lineages for all taxa show numerous independent lineages becoming isolated in springs; and (iii) biogeographic patterns in all taxa show reciprocal monophyly of individuals associated with different spring complexes as well as ancestral biogeographic states corresponding with surface drainages.

Our results support the hypothesis that the communities inhabiting this refugial environment act as an ‘evolutionary cohort’ where the community responds as a whole to environmental change through time. There are obvious biological differences between the invertebrate taxa endemic to the GAB springs (e.g. snails vs crustaceans, ostracods vs amphipods, aquatic vs semi-terrestrial isopods); despite this, the impact of a major climate shift from mesic to arid and the fragmentation of aquatic species into isolated ‘islands’ appear to have been more powerful than any life-history variable in shaping the evolutionary history and composition of the communities of desert spring endemics.

With all taxa demonstrating multiple lineages that extend back into the time before the formation of present-day deserts, the GAB springs have preserved significant diversity from a now extinct ecosystem. Even if only a small proportion of taxa survived the significant climatic shift from mesic to arid, and the subsequent contraction of their range into refugia, then the preserved diversity within the

springs is indicative that the Lake Eyre Basin had quite high species diversity during the Miocene. Alternatively, the GAB springs may have been colonized by species from a much broader range, although this seems unlikely given the very poor dispersal range of most of the species involved (Worthington Wilmer *et al.* 2008; Robertson *et al.* 2014). The results of this study complement previous findings of a rich vertebrate fauna in the region (e.g. lungfish, dolphins, large crocodiles and turtles), which infer warm conditions and a substantial body of permanent water (Martin 2006). In general, however there is little known about the aquatic fauna of this part of Australia through the Miocene, and the GAB springs have performed the critical *museum*-like role of preserving 'living fossils' from this time period.

This study also supports the hypothesis that a shift into isolated refugial habitat plays a major role in generating new diversity (Hewitt 2000). In addition to the preservation of lineages, isolation in spring refugia has clearly led to a large degree of speciation in all taxa. Intriguingly, the GMYC analyses suggest that the border between intra- and interspecific branching patterns in all taxa occurs in the same period as spring formation. As GMYC analyses resulted in a simplistic recognition of the species boundaries (Carstens *et al.* 2013), the results need to be interpreted with caution, but regardless of actual species-level diversity, it is clear that the time in refugia has acted as a *cradle* of significant diversification among the taxa examined.

Over recent times, speciation research has tended to highlight instances of ecological variation and selection in event of gene flow (Papadopoulos *et al.* 2011; Smadja & Butlin 2011) – despite the fact that geography is likely to be the predominant force in determining species differences (Coyne & Orr 2004). With the majority of these spring lineages limited to single catchments, biogeographic history and low dispersal ability have been the predominant forces shaping the evolution of endemic spring communities. Poor dispersal ability appears counter intuitive for species that persist in incredibly fragmented habitats (Reinhardt *et al.* 2005). However, dispersal-restricted species in climate refugia are not unexpected, as species with broader dispersal abilities are more likely to have simply shifted their distributions to more favourable habitats (Berg *et al.* 2010).

Our observations of a simple spring habitat, high levels of phylogenetic diversity in all lineages, lack of phenotypic diversification in lineages that have evolved within the springs (King 2009) and complete lack of sympatric sister lineages, suggest that the niche diversification has not occurred alongside evolutionary divergence in the GAB springs. Instead, it appears that phylogenetic niche conser-

vatism has had profound consequences for the build-up of species diversity in this habitat (Wiens 2004). In the face of a change from mesic to arid, niche conservatism, combined with low dispersal ability, would force these aquatic species to track their preferred habitats. This in turn has promoted vicariant fragmentation and formation of new evolutionary lineages, providing a neat explanation for the evolution of GAB spring diversity, where the formation of springs in central Australia appears to have led to multiple instances of non-adaptive radiations across many different taxa.

Freshwater ecosystems, particularly those that already promote habitat specialization such as alpine headwaters and arid river and lake systems, are likely to become increasingly fragmented in the face of future climate change. Despite the likelihood that these events will lead to some extinction, our results suggest that species which manage to survive within these habitats are likely to undergo lineage diversification, possibly to greater levels than existed previously. The caveat to any future diversification is that isolated and fragmented habitats need to remain viable for a very long period of time for this to occur. Small and isolated populations are prone to extinction events – the relative stability of a desert spring environment (i.e. with a constant supply of groundwater) appears to have allowed these species to persist. However, even these systems are now threatened by over extraction of water and invasive species (Murphy *et al.* 2013).

Our results support the model proposed earlier (Fig. 1), where climate refugia play a critical role as museums of diversity, preserving evolutionary history from an ecosystem that no longer exists. In addition, we have demonstrated refugia can play a critical role as evolutionary cradles, and are likely to drive the diversification of lineages into the future. It is important therefore not just to think the role of refuges from a species preservation point of view, but these generally isolated and fragmented habitats play a critical role in the generation of future biodiversity.

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References

- Alley, N. F. (1998). Cainozoic stratigraphy, palaeoenvironments and geological evolution of the Lake Eyre Basin. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 144, 239–263.
- Berg, M. P., Kiers, E. T., Driessen, G., Van Der Heijden, M., Kooi, B. W., Kuenen, F., Liefing, M., Verhoef, H. A. & Ellers, J. (2010). Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, 16, 587–598.
- Brower, A. V. Z. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Science USA*, 91, 6491–6495.
- Burbrink, F. T. & Pyron, R. A. (2011). The impact of gene-tree/species-tree discordance on diversification-rate estimation. *Evolution*, 65, 1851–1861.
- Byrne, M., Yeates, D. K., Joseph, L., Kearney, M., Bowler, J., Williams, M. A. J., Cooper, S., Donnellan, S. C., Keogh, J. S., Leys, R., Melville, J., Murphy, D. J., Porch, N. & Wyrwoll, K.-H. (2008). Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*, 17, 4398–4417.
- Carstens, B. C. & Richards, C. L. (2007). Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution*, 61, 1439–1454.
- Carstens, B. C., Pelletier, T. A., Reid, N. M. & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, 22, 4369–4383.
- Coyne, J. A. & Orr, H. A. (2004). *Speciation*. Sunderland, Massachusetts: Sinauer Associates.
- Davis, J., Pavlova, A., Thompson, R. & Sunnucks, P. (2013). Evolutionary refugia and ecological refuges: key concepts for conserving Australian arid zone freshwater biodiversity under climate change. *Global Change Biology*, 19, 1970–1984.
- Drummond, A. J. (2010) Geneious v5.3. Available from <http://www.Geneious.com>.
- Drummond, A. J. & Rambaut, A. (2007). Beast: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214.
- Fujioka, T., Chappell, J., Fifield, K. & Rhodes, E. (2009) Australian desert dune fields initiated with Pliocene–Pleistocene global climatic shift. *Geology* 37, 51–54.
- Guzik, M. T., Adams, M. A., Murphy, N. P., Cooper, S. J. B. & Austin, A. D. (2012). Desert springs: deep phylogeographic structure in an ancient endemic crustacean (*Phreatomerus latipes*). *PLoS ONE*, 7, e37642.
- Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405, 907–913.
- Johnson, J. B. (2002). Evolution after the flood: phylogeography of the desert fish Utah chub. *Evolution*, 56, 948–960.
- Keppel, G., Van Niel, K. P., Wardell-Johnson, G. W., Yates, C. J., Byrne, M., Mucina, L., Schut, A. G. T., Hopper, S. D. & Franklin, S. E. (2012). Refugia: identifying and understanding safe havens for biodiversity under climate change. *Global Ecology and Biogeography*, 21, 393–404.
- Ketmaier, V., Argano, R. & Caccone, A. (2003). Phylogeography and molecular rates of subterranean aquatic stenassellid isopods with a peri-Tyrrhenian distribution. *Molecular Ecology*, 12, 547–555.
- King, R. A. (2009). Two new genera and species of chiltoniid amphipods (Crustacea: Amphipoda: Talitroidea) from freshwater mound springs in south Australia. *Zootaxa*, 2293, 35–59.
- Kodric-Brown, A. & Brown, J. H. (2007). Native fishes, exotic mammals, and the conservation of desert springs. *Frontiers in Ecology and the Environment*, 5, 549–553.
- Lemey, P., Rambaut, A., Drummond, A. J. & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS Computational Biology*, 5, e1000520.
- Leys, R., Watts, C. H. S., Cooper, S. J. B. & Humphreys, W. F. (2003). Evolution of subterranean diving beetles (Coleoptera: Dytiscidae: Hydroporini, Bidessini) in the arid zone of Australia. *Evolution*, 57, 2819–2834.
- Liu, H. P. & Hershler, R. (2009). Genetic diversity and population structure of the threatened bliss rapids snail (*Taylorconcha serpenticola*). *Freshwater Biology*, 54, 1285–1299.
- Martin, H. (2006). Cenozoic climatic change and the development of the arid vegetation in Australia. *Journal of Arid Environments*, 66, 533–563.
- Murphy, N. P., Adams, M. & Austin, A. D. (2009). Independent colonization and extensive cryptic speciation of freshwater amphipods in the isolated groundwater springs of Australia's Great Artesian Basin. *Molecular Ecology*, 18, 109–122.
- Murphy, N. P., Breed, M. F., Guzik, M. T., Cooper, S. J. & Austin, A. D. (2012). Trapped in desert springs: phylogeography of Australian desert spring snails. *Journal of Biogeography*, 39, 1573–1582.
- Murphy, N. P., Adams, M., Guzik, M. T. & Austin, A. D. (2013). Extraordinary micro-endemism in Australian desert spring amphipods. *Molecular Phylogenetics and Evolution*, 66, 645–653.
- Papadopoulos, A. S., Baker, W. J., Crayn, D., Butlin, R. K., Kynast, R. G., Hutton, I. & Savolainen, V. (2011). Speciation with gene flow on Lord Howe Island. *Proceedings of the National Academy of Sciences*, 108, 13188–13193.
- Paradis, E., Claude, J. & Strimmer, K. (2004). Ape: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290.
- Ponder, W. (2002). Desert springs of the Australian Great Artesian Basin. Proceedings of the meeting on Spring-fed wetlands: important scientific and cultural resources of the intermountain region pp. 1–13.
- Ponder, W. F., Eggler, P. & Colgan, D. J. (1995). Genetic differentiation of aquatic snails (Gastropoda: Hydrobiidae) from artesian springs in arid Australia. *Biological Journal of the Linnean Society*, 56, 553–596.
- Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., et al. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55, 595–609.
- Prescott, J. R. & Habermehl, M. A. (2008). Luminescence dating of spring mound deposits in the southwestern Great Artesian Basin, northern South Australia. *Australian Journal of Earth Sciences*, 55, 167–181.
- R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rabosky, D. L. (2006). Laser: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evolutionary Bioinformatics Online*, 2, 247.
- Rambaut, A. & Drummond, A. J. (2007). Tracer v1.4 Institute of Evolutionary Biology. University of Edinburgh, Edinburgh, UK. Available <http://beast.bio.ed.ac.uk/software/tracer>

- Reinhardt, K., Köhler, G., Maas, S. & Detzel, P. (2005). Low dispersal ability and habitat specificity promote extinctions in rare but not in widespread species: The Orthoptera of Germany. *Ecography*, 28, 593–602.
- Robertson, H. L., Guzik, M. T. & Murphy, N. P. (2014). Persistence in the desert: ephemeral waterways and small-scale gene flow in the desert spring amphipod, *Wangiannachiltonia guzikae*. *Freshwater Biology*, 59, 653–665.
- Shepard, W. D. (1993). Desert springs—both rare and endangered. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 3, 351–359.
- Shoo, L. P., Hoffmann, A. A., Garnett, S., Pressey, R. L., Williams, Y. M., Taylor, M., Falconi, L., Yates, C. J., Scott, J. K., Alagador, D. & Williams, S. E. (2013). Making decisions to conserve species under climate change. *Climatic Change*, 119, 239–246.
- Smadja, C. M. & Butlin, R. K. (2011). A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology*, 20, 5123–5140.
- Wiens, J. J. (2004). Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution*, 58, 193–197.
- Wiens, J. J. & Donoghue, M. J. (2004). Historical biogeography, ecology and species richness. *Trends in Ecology & Evolution*, 19, 639–644.
- Wilke, T., Schultheiß, R. & Albrecht, C. (2009). As time goes by: a simple fool's guide to molecular clock approaches in invertebrates*. *American Malacological Bulletin*, 27, 25–45.
- Worthington Wilmer, J., Elkin, C., Wilcox, C., Murray, L., Niejalke, D. & Possingham, H. (2008). The influence of multiple dispersal mechanisms and landscape structure on population clustering and connectivity in fragmented artesian spring snail populations. *Molecular Ecology*, 17, 3733–3751.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Phylogenetic analyses for the 6 endemic taxa included in this study.

Fig. S2. Lineage through time (LTT) plot for each of the taxa colours correspond to each taxa.

Fig. S3. Ancestral reconstruction of surface catchments: phylogenetic analyses as per Figs 2 and S1.

Table S1. Sampling of taxa across the Lake Eyre spring complexes and catchments (Fig. 2).

Table S2. Results of diversification analyses.