Journal of Biogeography (J. Biogeogr.) (2015) 42, 2374–2388

ORIGINAL ARTICLE

Dispersal in the desert: ephemeral water drives connectivity and phylogeography of an arid-adapted fish

Krystina D. Mossop[1]*, Mark Adams[2,3], Peter J. Unmack[4], Katie L. Smith Date[1,5], Bob B. M. Wong[1] and David G. Chapple[1]

[1]School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia, [2]Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia, [3]School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia, [4]Institute for Applied Ecology and Collaborative Research Network for Murray-Darling Basin Futures, University of Canberra, Canberra ACT 2617, Australia, [5]Sciences Department, Museum Victoria, GPO Box 666, Melbourne, Victoria 3001, Australia

*Correspondence: Krystina D. Mossop, School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia.
E-mail: krystina.mossop@monash.edu

ABSTRACT

Aim We examine landscape processes shaping the range-wide phylogeography of a dispersal-limited, desert-dwelling fish (the desert goby, *Chlamydogobius eremius*) in arid Australia.

Location South-western Lake Eyre Basin, central Australia.

Methods We obtained sequence data for the mitochondrial cytochrome *b* gene ($n = 513$ individuals) and nuclear genetic markers (51 allozyme loci; $n \geq 128$ individuals) to investigate the phylogeographic relationships among 51 populations. Sampling spanned multiple habitat types (permanent desert springs, ephemeral rivers) and sub-catchments, and the entire distribution of *C. eremius* and representatives of its sister species, *Chlamydogobius japalpa*. Phylogeographic analyses (genetic diversity, AMOVA, Tajima's *D*, W_{ST}, mismatch distribution) were used to explore the distribution and partitioning of mtDNA variation; principal coordinates analysis and neighbour-joining tree networks were used to explore allozyme variation.

Results Three main genetic groups exist across *C. eremius*/*C. japalpa* populations. The geographical distributions of these groups reflected the historical and current confluence point of major rivers in the region. Surprisingly, permanent desert springs did not contain higher genetic diversity than ephemeral rivers.

Main conclusions Genetic structuring of *Chlamydogobius* populations revealed unanticipated levels of connectivity, indicating that the ephemeral waters of Lake Eyre have allowed gene flow across drainage boundaries and large distances. Phylogeographic breaks reveal that connectivity relies on temporary surface water, while rapid temporal changes in diversity highlight flood-driven dispersal as the main means of gene flow between localities and habitats. Dispersal pathways reveal that ecological context (life history and tolerance of extreme conditions) has played a key role in shaping observed patterns.

Keywords
allozymes, arid zone, biogeography, *Chlamydogobius eremius*, *Chlamydogobius japalpa*, cytochrome *b*, dispersal, freshwater fish, Lake Eyre Basin, phylogeography

INTRODUCTION

The extent of population genetic structuring within species can be strongly influenced by their capacity and tendency to disperse. However, barriers to dispersal can block or constrain the movement of individuals and the genes they carry. For freshwater species, terrestrial and marine habitats potentially restrict movement between disjunct freshwater patches (Unmack *et al.*, 2013), particularly for species with limited dispersal ability. As such, movement and life history traits can influence patterns of genetic structuring (Richardson, 2012). For example, fishes exhibit variation in connectivity that is associated with dispersal strategies (e.g. Sharma & Hughes, 2011; Young *et al.*, 2015). Similarly, in freshwater insects, the

http://wileyonlinelibrary.com/journal/jbi
doi:10.1111/jbi.12596

Journal of Biogeography

evolution of a more dispersive, winged life history stage can decrease genetic structuring across populations (Papadopoulou *et al.*, 2009).

In arid environments, environmental stochasticity can shape dispersal outcomes. In particular, unpredictable rainfall and high evaporation leave water sources highly variable in time and space. On the Australian continent, the onset of widespread aridification imposed heightened challenges to survival and dispersal for biota in the dwindling freshwater habitats (Byrne *et al.*, 2008). During the Cenozoic, peak warmth and humidity supported widespread rain forest and swamps, and a tropical fauna including lungfishes, flamingos and crocodiles (Fujioka & Chappell, 2010). However, a series of aridification events starting around 15 Ma saw a striking diversification of many lineages (e.g. lizards, Chapple & Keogh, 2004; amphipods and isopods, Cooper *et al.*, 2007; Byrne *et al.*, 2008). For freshwater taxa, water sources became increasingly rare, isolated, and ephemeral (Alley, 1998).

Today, arid and semi-arid climates characterize over 70% of the Australian continent (Fujioka & Chappell, 2010), and encompass the iconic Lake Eyre Basin. Covering one-sixth of the continent – an area of almost 1.2 million km^2 – the Lake Eyre Basin is one of the world's largest internal drainage systems. In the region surrounding its terminal point, the ephemeral salt lake known as Kati Thanda-Lake Eyre (hereafter 'Lake Eyre'; Fig. 1a), freshwater occurs in two main forms (Fensham *et al.*, 2011). First, a network of largely ephemeral rivers gives rise to spectacular but short-lived surface flows that support a boom-and-bust desert ecology (DeVogel *et al.*, 2004), and retract back to waterholes that are only rarely permanent (though more common in the Finke River). Second, permanent, groundwater-fed springs form fragmented aquatic 'oases' usually sustained by a vast aquifer of ancient water (the Great Artesian Basin; Gotch, 2013). Consequently, while springs *can* occur in close proximity to streams, or even intersect them, these potential refuges are not constrained by the location of other surface water features (Fig. 1). Moreover, springs are frequently elevated above surrounding landscapes and are potentially more isolated, a feature implicated in high levels of endemism within spring-dependent snails and amphipods (Ponder, 1986; Murphy *et al.*, 2012). However, the effect of spring isolation at broader spatial scales, or for more dispersive taxa, is largely unknown (but see Murphy *et al.*, 2010).

Importantly, as discrete water bodies occur here in a usually dry, arid matrix, floodwaters should be a key component of connectivity. This has two major implications. First, low rainfall, extreme evaporation rates and a flat landscape combine to produce the lowest and most variable annual run-off of any major drainage system globally (Kotwicki, 1986). Hence, while intense thunderstorms can create local flooding, extensive rainfall driven by a northern monsoon is usually required for rivers to reach Lake Eyre, which occurs as two branches: the larger Lake Eyre North, and the smaller, shallower Lake Eyre South (Costelloe *et al.*, 2005). At different rates (ranging from every two to 10 years on average, McMahon *et al.*, 2005), these desert rivers receive enough water to drain into the lake itself (either north or south, but not both) during filling events that potentially connect disparate river systems (Fig. 1a). Occasionally, extreme rainfall sends water through the narrow Goyder Channel (Fig. 1a) to connect the north and south branches of Lake Eyre; however, this has been recorded on only four occasions (Kotwicki, 1986).

A second implication is that while springs and rivers are hydrologically distinct, flooding potentially connects these two sources of water to each other, and both water sources to the terminal drainage points of Lake Eyre North and South (Fig. 1). Intriguingly, the connectivity afforded by the lake to fish populations is poorly known. Recent molecular work on four fish species found little evidence for contemporary gene flow between tributaries to Lake Eyre (Unmack, 2013). Differences in faunal assemblages between tributaries (Wager & Unmack, 2000) also suggest that for some species, the lake prohibits inter-catchment movement. Potential barriers result from the temperature, salinity and dissolved oxygen extremes observed within the lake's interior (Glover, 1971), exacerbated by the infrequency of synchronized flow events across multiple tributaries required for dispersal between rivers, via Lake Eyre (Unmack, 2013). In this context, stochastic flooding, permanent springs and a central confluence of drainages all potentially contribute to the connectivity of freshwater biota. The desert goby (*Chlamydogobius eremius*, Zietz 1896; Gomon & Bray, 2011) is an ideal species for investigating consequences of the Lake Eyre region's dynamic aquatic connectivity. A small (< 6 cm) benthic fish, *C. eremius* occurs in a broad arc spanning the western and southern margins of Lake Eyre, as well as the Warburton River in the north-east (Fig. 1). Three key traits distinguish *C. eremius* from other local aquatic fauna with regard to dispersal potential and the nature and distribution of possible habitat. First, while more mobile than many aquatic invertebrates, the species has a relatively poor swimming ability for a fish (McNeil & Schmarr, 2009). Furthermore, as larvae, *C. eremius* rapidly settles onto the substrate and does not experience the pelagic larval phase that facilitates dispersal in many other fishes (e.g. McGlashan & Hughes, 2001). Second, physiological and behavioural adaptations allow *C. eremius* to tolerate extreme fluctuations in water quality (Glover, 1982; Thompson & Withers, 2002), including salinities threefold that of seawater (we have recorded fish in water > 100,000 mg/L). Third, the species inhabits both springs and rivers, and thus both permanent and temporary habitats (Fig. 1).

We used mitochondrial DNA (mtDNA) sequence and allozyme data to investigate the legacy of a complex biogeographical history on population genetic structuring in *C. eremius*. Comprehensive sampling across the Lake Eyre Basin enabled us to encapsulate important environmental variation at both large (e.g. between separate drainages) and

Figure 1 Map of 51 sampled *Chlamydogobius* populations in the Lake Eyre Basin, with inset of the location of the study region (in orange) and the Great Artesian Basin (in dark grey) within the Australian continent. Location codes in (a) correspond to sample sets listed in Table 1 and show the location of sampled rivers and springs. The boundaries and names of five sub-catchments relevant to Lake Eyre are depicted in orange; all but the Cooper Creek contain *Chlamydogobius* populations. Individual pie charts illustrate the cytochrome *b* haplotypes detected in sample sets, depicted by (b) past and (c) current sampling periods. Pie chart sizes are proportional to the frequency of individuals per sample set, and spring populations are denoted with a black border; river populations have a grey border. A legend of colour coding of haplotypes is provided in (d), where '*h*' refers to the 26 individual cytochrome *b* haplotypes. Grey brackets depict the geographical location of the three genetic groups. In (b), ★ denotes the location of 'disjunction 1', as referred to in text; ★ denotes the location of 'disjunction 2'. NB: some geographical locations have been adjusted slightly for illustration purposes.

smaller spatial scales (e.g. between habitat types). Historical samples allowed a rare opportunity to investigate the consistency of phylogeographic patterns over time. We addressed three hypotheses. First, drainage divides are traditionally important for freshwater connectivity (Hughes *et al.*, 2013); for *C. eremius*, increasing aridification and the historical loss of a permanent confluence point (a perennial Lake Eyre) have potentially left drainages functionally isolated. In this case, we expect sub-catchments (Fig. 1a) to drive population genetic structuring. Second, the fact that Lake Eyre does intermittently contain water, however harsh its conditions, leads to the hypothesis that the lake allows connectivity among its tributaries. Here, we would predict increased connectivity, and thus reduced structuring, among fish populations in different sub-catchments. Lastly, since permanent habitats could provide refuges for aquatic organisms, a third hypothesis is that (a) springs are more isolated habitats and gene flow is higher among connected river patches (Davis *et al.*, 2013), resulting in spring-river structuring, or (b) springs decrease structuring among populations, for example, by acting as 'stepping stones' (Baguette *et al.*, 2013). In the former scenario, this habitat-level distinction intuitively lends itself to predictions about genetic diversity, namely, that permanent springs contain more diversity than ephemeral rivers.

MATERIALS AND METHODS

Tissue samples

Chlamydogobius eremius samples were sourced by field collection and frozen tissues held at the South Australia Museum. We also included representative samples of the closely related Finke goby (*Chlamydogobius japalpa*), an endemic of the adjacent Finke River system (Fig. 1a). While their original taxonomy was separated on the basis of fine meristic differences, the two species were not allozymically distinct (Adams, unpublished; cited in Larson, 1995), thus warranting further molecular assessments of their taxonomic status. Remaining tissue samples from our contemporary sampling were deposited at the South Australian Museum or Museum Victoria, and can be identified by their 'sample set' code (Table 1). 'Sample sets' comprised fish collected from a single geographical location, usually within a 2-year period.

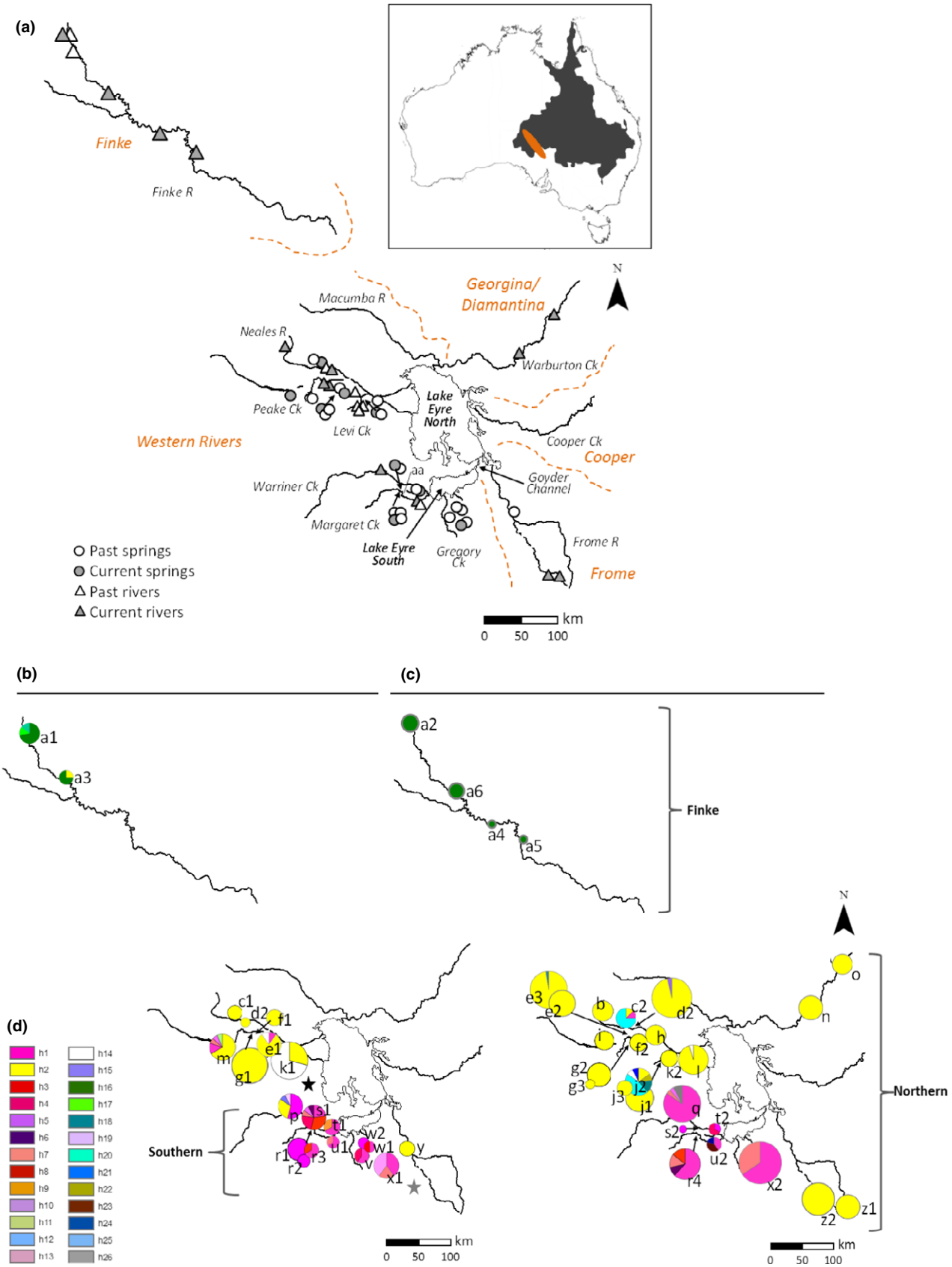
Mitochondrial DNA

Sampling

A total of 513 *Chlamydogobius* individuals were selected to fully span both the species range and the breadth of inhabited habitats, namely, spring, bore and river environments (Table 1, Fig. 1). This resulted in sampling from 31 localities in the region surrounding Lake Eyre (see Table S1 in Appendix S1 of Supporting Information). Samples comprised 50 'sample sets', which averaged 10.3 individuals per sample set (Table 1). Throughout, bores were pooled with springs due to their ecological similarity (our unpublished data).

DNA isolation, amplification and sequencing

Total DNA was obtained from ~0.25 cm^3 of caudal fin or muscle using DNeasy Blood and Tissue Extraction Kits (Qiagen Inc., Hilden, Germany). We amplified the second half of the cytochrome *b* (Cyt*b*) gene (560 bp per individual), as previous work had shown useful levels of variability in *Chlamydogobius* (P.J. Unmack, unpublished data). Samples were amplified using the primers goby505F (5′-TCAGTTGACAATGCCACCCT-3′) and EleoThr40 (5′-GATTTTAACCTCCTGCGTCCG-3′). Volumes for polymerase chain reaction (PCR) components per 25-μL reaction were: 1 μL template DNA, 1 μL of each 10 μM primer and Taq DNA polymerase via 12.5 μL of Promega GoTaq 2× Colourless Mastermix. Amplification parameters were: 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 20 s, 72 °C for 50 s and a final extension step at 72 °C for 5 min. PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA). We sent the purified products to Macrogen (Seoul, Korea) for DNA sequencing using an ABI 3730XL capillary sequencer. Sequences were edited and aligned (using default parameters of Clustal W; Yang *et al.*, 2013) in Geneious 6.2 (Kearse *et al.*, 2012). We translated all coding region sequences to ensure none contained premature stop codons. All unique sequence data were deposited



in GenBank, accession numbers KP146114-KP146139 (see Table S2 in Appendix S1).

Phylogeographic analyses: molecular diversity and population structure

Using DnaSP 5.10 (Librado & Rozas, 2009), we estimated within-population levels of genetic diversity (number of haplotypes, *h*; haplotypic diversity, *Hd*; number of polymorphic sites, S; nucleotide diversity, π). We used Network 4.612 (Bandelt *et al.*, 1999) to create maximum parsimony networks of Cyt*b* haplotypes, using statistical parsimony with a 95% probability of no multiple substitutions. We calculated pairwise W_{ST} values (an analogue of Wright's fixation index F_{ST}) in Arlequin 3.5.1.3 to estimate among-population differentiation. In Arlequin, we performed hierarchical analysis of molecular variance (AMOVA) to investigate the partitioning of genetic variation within and among populations and regions, and to test the contributions of several landscape factors (by allowing the default scenario of drainage separations to be modified by habitat type and/or Lake Eyre, to correspond to our ecological predictions). AMOVAs therefore explored: (1) the individual sub-catchments in which sites were located (see Fig. 1); (2) detailed drainage configuration (distinguished from (1) in its categorization of sites based on their spatial configuration and the river systems in which they occurred; see Table 1); (3) Lake Eyre connectivity: whether sites within sub-catchments were associated with drainage into Lake Eyre North or South; and (4) habitat type (spring or river). AMOVA and W_{ST} analyses followed methods in Chapple *et al.* (2012). We explored the exclusion of sites with < 7 sequenced individuals in AMOVAs, however, retained the full data set upon finding that this did not affect the outcomes. We used two-factor ANOVAs in Systat 13 (Systat Software, Inc., 2013) to test for a difference in genetic diversity (*h*, *Hd* and π) between habitats, and between major groups best supported by AMOVAs. Using Arlesquin we calculated Tajima's *D* (Tajima, 1989), Fu's F_S statistic (Fu, 1997) and mismatch distributions to test for signatures of population expansion (significance levels were calculated by 10,000 permutations). Details for interpreting Tajima's *D* and Fu's F_S statistic values, along with the use of mismatch frequency histograms, and raggedness index (RI) and sum of squared deviations (SSD) calculations, are provided in Chapple *et al.* (2012). These statistics, and W_{ST} values, were calculated for populations with $n \geq 5$.

Temporal comparisons

We used two approaches to investigate the effect of time on the distribution of mtDNA variation. First, to investigate the temporal stability of overall phylogeographic patterns, we explored Cyt*b* sequence variation by separating sample sets into 'past' and 'current' sampling windows, based on the time of collection, and occasionally, knowledge of ecological change at a site (e.g. flood or disturbance). This resulted in 'past' (21.5 ± 4.4 years old; generally collected between 1984 and 1996) and 'current' (4.2 ± 4.0 years old; collected between 2000 and 2014) data sets, which were separated in time by up to 30 years, and included known flood events in different local areas (e.g. southern region: $n \geq 9$; northern region: $n \geq 10$; B. Backway, unpublished data). As *C. eremius* matures rapidly and produces at least two generations in a single breeding season (Glover, 1971), this interval equates to between 30 and 60 generations and is thus biologically meaningful. We compared the results of AMOVAs run separately for past and current data sets to look for discrepancies in the factors underlying population genetic structuring. For *C. eremius* only, we used general linear models in Systat 13 (Systat Software, Inc., 2013) to test for statistically significant differences in genetic diversity measures (*h*, *Hd*, π) between sampling windows for populations with $n \geq 5$ samples. For this test, 'current', Northern populations were better represented than other groups, so we took a blind, random subsample to enable analysis of groups with equal sample sizes. Second, as a subset of *C. eremius* localities was sampled at multiple time points, we conducted intra-site comparisons of genetic variation to explore potential localized shifts within populations. Cyt*b* haplotypic variation was first compared visually. For each of the seven sites with adequate sample sizes and heterogeneity across different temporal replicates, we used genepop 3.1b (Rousset, 2008) to determine whether the observed differences were statistically significant following sequential Bonferroni correction for multiple tests (Rice, 1989).

Isolation by distance

As dispersal in *Chlamydogobius* is most likely flood-mediated, we explored partial correlations between genetic and spatial distances for a subset of localities (those within the species' core range, on the western side of Lake Eyre), to test for an isolation-by-distance pattern in population genetic structure (Hammer *et al.*, 2013). To construct distance matrices, river and straightline geographical distances were estimated using the path function in google earth 6.0.3.2197 (Google Inc., 2011), based on likely flow patterns that could arise via temporary creeks between sites (Robertson *et al.*, 2014). Although we hypothesized that any role of isolation-by-distance would likely occur via riverine distance, geographical distance was included to control for its possible effect (Diniz-Filho *et al.*, 2013). We used partial Mantel tests (10,000 permutations) in Arlequin to investigate whether river and geographical distance matrices helped predict genetic distances (i.e. W_{ST} values; Legendre & Legendre, 2012).

Allozymes

Sampling

Our principal allozyme data set comprised $n = 128$ individuals (116 *C. eremius* and 12 *C. japalpa*; Table 1) at 51 putative allozyme loci. For some analyses, we included an additional

Table 1 Background information for *Chlamydogobius eremius/C. japalpa* populations (by sample set) used in mtDNA and allozyme analyses. Further locality data are provided in Table S1 in Appendix S1.

Sample set	Locality name	Habitat type	Sampling year	Sampling period	*n* allozymes [*]	*n* Cyt*b*	Cyt*b* haps present[n]	River system†	Lake Eyre connection‡
a1	Finke R, Glen Helen Gorge	River	1992	Past	3 [5]	7	16^{5}, 17^{1},18^{1}	Finke	None
a2	Pioneer Creek	River	2001	Current	6	6	16	Finke	None
a3	Finke R, Hermannsburg	River	1992	Past	1 [5]	4	2^{1}, 16^{3}	Finke	None
a4	Finke R, Idracowra	River	2001	Current	1	1	16	Finke	None
a5	Finke R, Horseshoe Bend	River	2001	Current	1	1	16	Finke	None
a6	Finke R, Henbury	River	2014	Current	–	5	16	Finke	None
b	Stewart WH	River	2002	Current	5	7	2	Neales	North
c1	Ockenden Spring	Spring	2000	Past	4	4	2	Neales	North
c2	Ockenden Spring	Spring	2012	Current	–	8	1^{1}, 2^{1}, 20^{6}	Neales	North
d1	Algebuckina WH	River	2000	Past	3	2	2	Neales	North
d2	Algebuckina WH	River	2011–13	Current	–	28	2^{27}, 15^{1}	Neales	North
e1	Peake Creek	River	1992	Past	4 [5]	10	1^{1}, 2^{8}, 14^{1}	Peake	North
e2	Peake Creek	River	2000–02	Current	9	13	2	Neales	North
e3	Peake Creek	River	2009	Current	–	26	2^{25}, 25^{1}	Neales	North
f1	North Freeling Springs	Spring	1996	Past	5	5	2	Neales	North
f2	North Freeling Springs	Spring	2012	Current	–	6	2	Neales	North
g1	Freeling Springs	Spring	1994	Past	5	29	2	Neales	North
g2	Freeling Springs	Spring	2000	Current	3	3	2	Neales	North
g3	Freeling Springs	Spring	2012	Current	–	12	2	Neales	North
h	Tardetakarinna WH	River	2002	Current	5	7	2	Neales	North
i	Cardajalburrana Spring	Spring	2002	Current	9	8	2	Neales	North
j1	Levi Creek	River	2011	Current	–	4	2	Peake	North
j2	Levi Creek	River	2012	Current	–	13	2^{2}, 14^{1}, 19^{2}, 20^{6}, 21^{1}, 22^{1}	Peake	North
j3	Levi Creek	River	2014	Current	–	14	2	Peake	North
k1	The Fountain	Spring	1994–96	Past	10	31	2^{8}, 14^{23}	Peake	North
k2	The Fountain	Spring	2014	Current	–	7	2	Peake	North
l	Johnsons Bore	Bore	2013	Current	–	21	2^{20}, 14^{1}	Peake	North
m	Nilpinna Springs	Spring	1984	Past	4 [14]	15	1^{2}, 2^{10}, 5^{1}, 7^{1}, 11^{1}	Neales	North
n	Cowarie Dam	River	2012	Current	–	10	2	Warburton	North
o	North Ultoomurra WH	River	2002	Current	5	8	2	Warburton	North
p	Nunns Bore	Bore	1992	Past	4 [3]	13	1^{7}, 2^{4}, 12^{1},13^{1}	Warriner	South
q	Warriner Creek	River	2011	Current	–	26	1^{22}, 4^{1}, 7^{1}, 26^{2}	Warriner	South
r1	Coward Springs	Spring	1992	Past	4 [4]	10	1	Margaret	South
r2	Coward Springs	Spring	1996	Past	3	3	1	Margaret	South
r3	Coward Springs	Spring	2000	Past	3	3	1^{1}, 3^{2}	Margaret	South
r4	Coward Springs	Spring	2012–13	Current	–	21	1^{13}, 3^{3}, 6^{2},7^{3}	Margaret	South
s1	Coward Springs Railway Bore	Bore	1984	Past	4 [13]	13	1^{3}, 3^{4}, 4^{3}, 5^{1}, 6^{1}, 7^{1}	Margaret	South
s2	Coward Springs Railway Bore	Bore	2000	Current	1	1	1	Margaret	South
t1	Emerald Spring	Spring	1989	Past	1 [4]	6	1^{4}, 9^{2}	Warriner	South
t2	Emerald Spring	Spring	2000	Current	3	3	1^{1}, 4^{2}	Warriner	South
u1	Margaret Creek	River	2000	Past	3	3	1^{2}, 7^{1}	Margaret	South
u2	Margaret Creek	River	2012	Current	–	5	1^{2}, 23^{2},24^{1}	Margaret	South
v	Bopeechee Spring	Spring	1996	Past	5	5	1^{3}, 4^{2}	Finniss	South
w1	Dead Boy Spring at Hermit Hill	Spring	1995	Past	2	2	1^{1}, 8^{1}	Finniss	South
w2	Dead Boy Spring at Bopeechee	Spring	1996	Past	2	2	1^{2}	Finniss	South

Table 1 Continued

Sample set	Locality name	Habitat type	Sampling year	Sampling period	*n* allozymes [*]	*n* Cyt*b*	Cyt*b* haps present[n]	River system†	Lake Eyre connection‡
x1	Finniss Creek	River	1992	Past	[4]	10	1^4, 7^2, 10^4	Finniss	South
x2	Finniss Creek	River	2012–13	Current	–	35	1^{23}, 7^{12}	Finniss	South
y	Hergott Spring	Spring	1992	Past	2 [4]	5	2	Frome	North
z1	Leigh Creek	River	2003	Current	5	10	2	Frome	North
z2	Leigh Creek	River	2012	Current	–	22	2	Frome	North
aa	The Bubbler	Spring	2000	Current	3	–	–	Margaret	South

n = sample size, WH = waterhole, R = river.

*Indicates samples run in 1992 and cited in Larson (1995).

†Describes specific 'drainage' area designation used in AMOVA analyses.

‡Describes the most likely mode of connection between a locality and modern Lake Eyre, where 'North' indicates Lake Eyre North connectivity, 'South' indicates Lake Eyre South connectivity.

61 individuals (51 *C. eremius* and 10 *C. japalpa*) genotyped for 37 loci during an earlier allozyme study used in the taxonomic revision that split desert gobies into five congeners (Larson, 1995).

Allozyme analysis

Allozyme electrophoresis of muscle homogenates used cellulose acetate gels (Cellogel, MALTA, Milan) and standard protocols (Richardson *et al.*, 1986). After histochemical staining, the following enzymes or non-enzymatic proteins exhibited allozymically interpretable banding patterns: ACON, ACP, ACYC, ADA, ADH, AK, ALD, CA, CK, ENOL, EST, FDP, FUM, G6PD, GAPD, GLO, GOT, GP, GPD, GPI, GSR, IDH, LDH, MDH, ME, MPI, NDPK, NP, PEPA, PEPC, PEPD, PGAM, 6PGD, PGK, PK, PGM, TPI and UGPP. Full enzyme names, electrophoretic conditions, stain recipes, and allozyme nomenclature are presented elsewhere (Richardson *et al.*, 1986; Hammer *et al.*, 2007).

As advocated for allozyme data sets (Adams *et al.*, 2011), we initially used principal coordinates analysis (PCoA) to explore the genetic affinities among individuals, independent of their taxon, geographical location, or mtDNA profile. The methodological details for PCoA are presented in Hammer *et al.* (2007), while Adams *et al.* (2014) provide rationales for delineating genetic groups and deciding whether such groups represent candidate species or discrete subpopulations.

Having identified major groups, we undertook within-site assessments of the raw genotypes, using GENEPOP 3.1b, to check for statistically significant instances (after correcting for multiple tests) of linkage disequilibrium or departures from Hardy–Weinberg expectations. As none were detected, we calculated allele frequencies and pairwise genetic distances (both unbiased Nei's distance and the number of 'fixed' allozyme differences) for individual sample points, and the two *Chlamydogobius* species. Genetic affinities among sample sets were then assessed by constructing a neighbour-joining tree network from the Nei's *D* matrix. Hammer *et al.* (2007) provide all methodological details for these procedures.

RESULTS

Mitochondrial DNA

The edited Cyt*b* alignment contained 560 characters and 28 variable sites, revealing 26 distinct haplotypes within *C. eremius*/*C. japalpa* (Fig. 2). The geographical distribution of haplotypes indicated that there was a spatial component underlying their structure. Alongside rarer haplotypes, there were three main, common haplotypes: haplotypes 1 and 2 (separated by four base pairs), and haplotype 16 (Fig. 2). The most divergent of these, Haplotype 16, occurred only in *C. japalpa* samples, and was separated by the remaining two types by 12 and 10 base pairs respectively. Haplotypes 1 and 2 had a strong geographical basis: 96.2% of haplotype 1 individuals came from southern localities, and 98.2% of haplotype 2 individuals originated from northern localities. Three groups were thus identified, hereafter referred to as the Finke, Northern and Southern groups (Fig. 1). Pairwise W_{ST} values confirmed that the most substantial genetic differentiation occurred between genetic groups (Northern versus Southern = 0.750, Northern versus Finke = 0.936, Southern versus Finke = 0.859). W_{ST} levels among subcatchments were highly variable (ranging from 0.00 to 0.963; see Table S3 in Appendix S2 for pairwise values of sample sets). In AMOVAs, sorting populations by subcatchment alone yielded reasonable predictive power, but analyses that separated spatially associated springs and rivers only explained genetic variation when genetic group was included (Table 2). The most powerful configuration was a hierarchical one that first considered presence or absence of current connectivity to Lake Eyre, and second, whether populations were hydrologically connected to Lake Eyre North or South (Table 2). This structure aligned with the geographical separation of the three genetic groups. Based on Tajima's *D*, Fu's F_S and mismatch distributions, there was

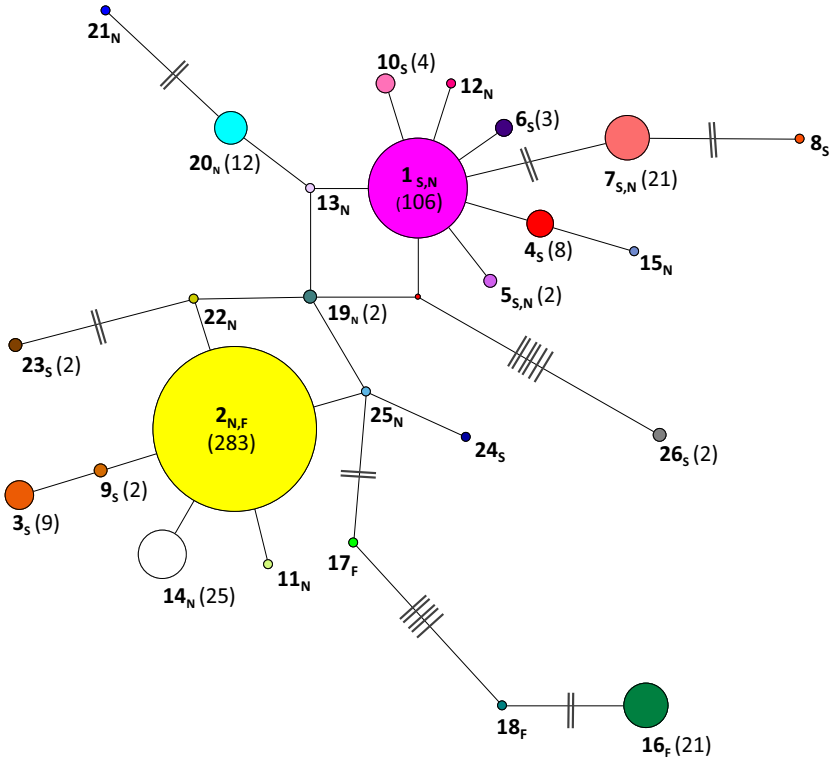


Figure 2 A maximum parsimony haplotype network of *Chlamydogobius* cytochrome *b* haplotypes in the Lake Eyre Basin illustrating the presence of three main genetic groups. Each circle represents a single haplotype, labelled with a unique number, while the size and bracketed value of each node reflect the number of individuals detected with that haplotype. Letters provided in subscript indicate the groups in which each haplotype was detected (F = Finke, N = Northern, S = Southern). Colour coding follows Fig. 1. Lines intersecting network branches indicate the number of mutations that separate adjacent haplotypes (branches not to scale). Intersecting lines or the absence of bracketed numbers indicate values of 1.

little evidence of population expansion (Table S1 in Appendix S1).

Isolation by distance

Given the strong structure between genetic groupings, and that sampling was most extensive across the western and southern sides of Lake Eyre (in which springs and inhabited rivers are most numerous), partial Mantel tests were conducted within this region only and independently for the Northern and Southern groups, to identify any secondary role of isolation-by-distance. There was no correlation between river distance and genetic distance for either the Northern ($r^2 = 0.01$, $P = 0.38$) or Southern ($r^2 < 0.01$, $P = 0.56$) populations, with distance matrices together explaining < 1% of the variation between populations.

Table 2 Analysis of molecular variance (AMOVA) at organizational levels of interest (and corresponding hypothesis tested) for mtDNA sequence variation in *C. eremius/C. japalpa* populations in the Lake Eyre region.

		Amount of variation explained (%)			
Level of organization	Tests which hypothesis?	Among groups* (d.f.)	Among populations within groups** (d.f.)	Within populations*** (d.f.)	*P*
Sub-catchment	1	50.49 (3)	34.76 (46)	14.75 (462)	All < 0.001
Drainage	1	70.27 (7)	9.34 (42)	20.39 (462)	All < 0.001
Sub-catchment + Lake Eyre connectivity	2	75.53 (4)	7.82 (45)	16.65 (462)	All < 0.001
Sub-catchment + Lake Eyre connectivity (PAST data set only)	2	71.91 (3)	6.01 (17)	22.08 (161)	All < 0.001
Sub-catchment + Lake Eyre connectivity (CURRENT data set only)	2	77.91 (4)	8.93 (24)	13.16 (301)	All < 0.001
Habitat type† (springs and rivers)	3	0 (1)	77.60 (48)	22.40 (462)	*0.59 **< 0.001 ***< 0.001
Habitat type + Lake Eyre connectivity†	3	62.66 (3)	12.55 (34)	24.79 (388)	All < 0.001

†Analyses confined to populations on the western side of Lake Eyre only, due to general absence of springs in other regions.

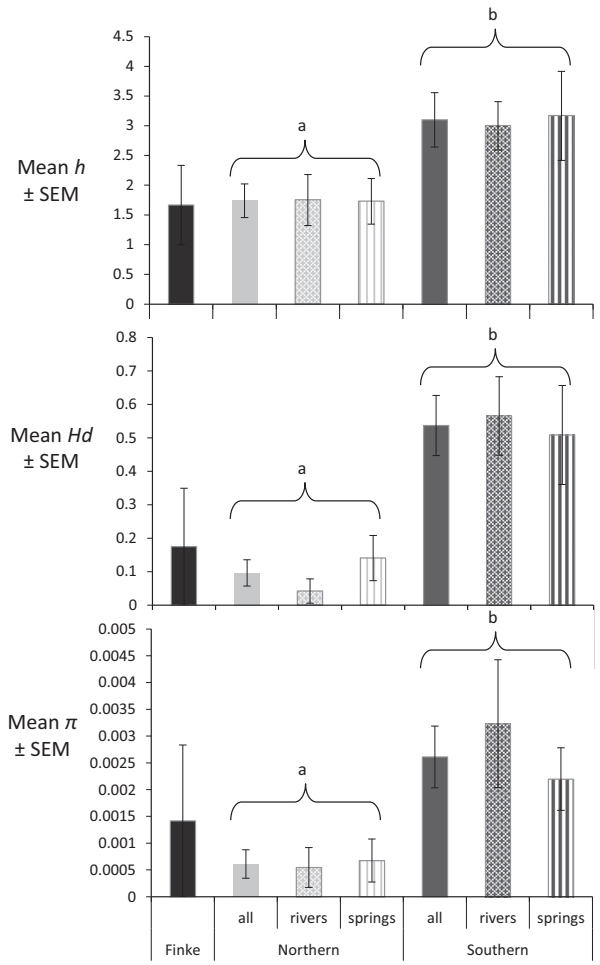


Figure 3 Comparison of mean estimates of cytochrome *b* sequence diversity for *Chlamydogobius* sample sets. Data are presented by genetic group and habitat type, and for each diversity measure, letters depict statistically significant differences among groups (where a ≠ b), as per ANOVAs. Finke River sample sets are shown for comparison, but were excluded from statistical analysis due to unequal sample sizes. *h* = number of haplotypes, *Hd* = haplotypic diversity, π = nucleotide diversity.

Genetic diversity

ANOVAs of *h*, *Hd* and π showed that the Southern group was more diverse than the Northern group (Fig. 3). Within each *C. eremius* group, however, there was no effect of habitat type (Fig. 3). We did observe a number of rare or genetically distinct haplotypes, e.g. Haplotypes 26 and 8, at sites *x* and *y* respectively (Table 1, Fig. 2). Additionally, there were apparent sub-catchment-related patterns in diversity within the Northern group. Here, only the Neales/Peake system, on the western side of Lake Eyre, contained any diversity; the two inhabited sub-catchments on the eastern side were monomorphic (Table 1, Fig. 1).

Temporal comparisons

Generally, there was limited evidence that time (past versus current) affected either population genetic structuring (Table 2, Fig. 1) or diversity (comparison of time points for *h* estimates: $F_{1,20} = 0.02$, $P = 0.89$; *Hd* estimates: $F_{1,20} = 0.08$, $P = 0.79$; π estimates: $F_{1,20} = 0.31$, $P = 0.59$) in *C. eremius*, suggesting that the overarching patterns were consistent, at least over the last ~30 years. However, some *C. eremius* populations underwent shifts in genetic diversity, with significant temporal contrasts seen within four localities (sites c, j, k and x; Fig. 4). For instance, repeated sampling over four years at Levi Creek (sample sets j1–j3) showed that this locality underwent rapid changes in its haplotypic makeup. The population shifted from being exclusively dominated by one haplotype (albeit within a limited sample size), to containing six haplotypes following localized flooding (Fig. 4). Complete drying of the location subsequently resulted in total loss of diversity.

Allozymes

An initial PCoA of the principal allozyme data set (128 individuals, 51 loci; Fig. 5) displayed the same fundamental phylogeographic pattern as the Cyt*b* analyses. The first PCoA dimension generally showed a primary split between individuals from most Southern group sites, and those from sites mostly featuring Northern or Finke haplotypes. The second PCoA dimension refined this overarching pattern to ultimately assign all but three individuals (those from Southern sample sets t1 and w2) into four phylogeographic groups, namely 'pure' southern, 'pure' northern, Finke and 'Freeling' (herein defined as sites g1 and g2). Each group was characterized by major differences ($50\% < \Delta p < 100\%$) in allele frequency at least one of three polymorphic loci (*Ck*, *Fum*, and *Idh1*; see Table S4 in Appendix S2), and also appeared in an additional PCoA of the extended allozyme data set (189 fish at 37–51 loci; analysis not presented).

The analyses based on sample sets also showed general support for the integrity of these four allozyme groups. A neighbour-joining network of sample sets represented in the extended allozyme data set (and based on unbiased Nei's distances, Table S4) allocated all sample sets but one (w2) into their expected groups (see Fig. S1, Appendix S2). None of the five sites (e, g, r, s, and t) represented by both 'past' versus 'present' sample sets displayed any statistically significant changes in allele frequency over time.

Other allozyme results were also consistent with the Cyt*b* outcomes. Overall levels of within-sample set variation were low (average observed heterozygosity (H_O) = 0.028, range: 0.000–0.093; Table S4). There was little support for a simple taxonomic dichotomy between populations currently assigned to either *C. eremius* or *C. japalpa* (Fig. 5 & Fig. S1 in Appendix S2).

DISCUSSION

Major biogeographical patterns

Hypothesis 2 – wherein connection to Lake Eyre changes connectivity among sub-catchments – had the strongest

	Northern group			Southern group			
Locality name	Ockenden Spring	The Fountain	Levi Creek	Emerald Spring	Coward Springs	Finniss Creek	Margaret Creek
Time 1							
Sample set (*n*)	c1 (4)	k1 (31)	j1/j3 (18)	t1 (6)	r1 (10)	x1 (10)	u1 (3)
Time 2							
Sample set (*n*)	c2 (8)	k2 (6)	j2 (13)	t2 (3)	r4 (21)	x2 (35)	u2 (5)
Probability test *P* value^	**0.0098***	**0.0008****	**0.0001*****	0.160	0.2	**0.002***	0.487

^Calculated in GENEPOP 3.1b (Rousset 2008), followed by Bonferonni correction procedure. Bold *P* values indicate statistical significance. **P*_adjusted < 0.05, ***P*_adjusted < 0.01, ****P*_adjusted < 0.001

Figure 4 Visual representation and statistical comparison of cytochrome *b* haplotypes detected in repeatedly sampled *C. eremius* localities that showed sequence heterogeneity. Pie charts reflect colour coding provided in Fig. 1. Populations (as sample sets) are designated by genetic group and time frame (time 1 or time 2). Sample set codes and corresponding sample sizes (*n*) are provided.

support for explaining genetic structuring in *C. eremius*. Overarching patterns were consistent with a Stream Hierarchy Model (Hammer *et al.*, 2013), in which genetic structuring was first explained by whether a region had contemporary or only historical connectivity with Lake Eyre: a difference reflected in the distinctiveness of the Finke River *Chlamydogobius* (Fig. 2). The second hierarchical level of structure reflected the mode of current connection, i.e. whether populations occurred in sub-catchments that flow into Lake Eyre North or South. This distinction corresponded to the geographical separation of the two *C. eremius* groups, including a striking disjunction in the south-east ('disjunction 2', Fig. 1a). Dry-land barriers that match this framework, and not necessarily traditional drainage boundaries, delineate the distributions of the three, genetically distinct groups identified by both mtDNA and allozyme results (Figs 2 & 5). Indeed, counter to the prediction that populations have been isolated by sub-catchment alone (hypothesis 1), as occurs for other freshwater fishes (Faulks *et al.*, 2010; Hughes *et al.*, 2013), *C. eremius* clearly has undertaken movement between rivers via Lake Eyre to produce surprising phylogeographic patterns. The broad geographical nature of the between-river genetic exchange indicates that large surface water flows facilitate extensive gene flow: a picture that contrasts with the Death Valley Model (DVM) of structuring typified by ecologically similar desert pupfishes of arid North America, in which populations trapped in remnant habitats have undergone substantial genetic divergence (Meffe & Vrijenhoek, 1988; Duvernell & Turner, 1999).

A strong signature of Lake Eyre in the distribution of genetic groups also suggests that major barriers to gene flow arose only when the lake reached its current, entirely ephemeral form (around 50,000 years ago; Habeck-Fardy & Nanson, 2014). Prior to this, dry phases were interrupted by wetter periods, including a prolonged freshwater phase around 85,000–100,000 years ago (Magee *et al.*, 2004). This geological scenario concords broadly with estimates of the Finke River becoming isolated from Lake Eyre around 10,000–13,000 years ago via sand dune formation (Bowler & Wasson, 1984), although other data suggest that more recent palaeofloods connected the two within the last several thousand years (Pickup *et al.*, 1988). Indeed, while *C. japalpa* samples did comprise the most distinctive Cyt*b* haplotypes, genetic overlap at both allozymic and mtDNA loci confirm that molecular divergence between *C. eremius* and the Finke River-restricted *C. japalpa* is no deeper than that typically seen at an intraspecific level. Intriguingly, this matches broadly with hardyhead fish congeners (*Craterocephalus* spp.) similarly confined to the Finke and Lake Eyre regions (Adams *et al.*, 2011). In both cases, morphological criteria differentiate between a widespread (*C. eremius*, *Craterocephalus eyresii*) and a localized (*Chlamydogobius japalpa*, *Craterocephalus centralis*) species (Crowley & Ivantsoff, 1990; Larson, 1995), but molecular genetic structure does not. Indeed, haplotype 2, which is otherwise associated with the Northern group, was detected in one Finke River *Chlamydogobius* individual. While the sharing of identical Cyt*b* haplotypes could represent shared ancestral variation, an

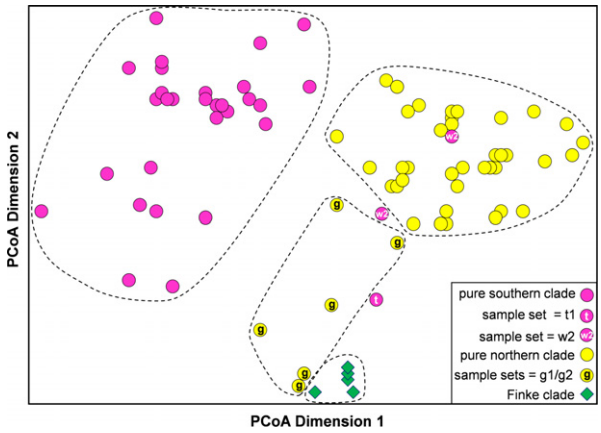


Figure 5 Principal coordinates analysis of the 128 *Chlamydogobius* individuals included in the principal allozyme study. Individuals are identified by a symbol depicting their group, according to the included legend. Sample sets t1 and w1 (Emerald Spring and Dead Boy Spring) are labelled to reflect individuals from those populations that did not fall into the main groups. The relative PCoA scores have been plotted for the first and second dimensions, which individually explained 21% and 17% respectively of the total multivariate variation present. Envelopes highlight the four groups.

alternative explanation is an older isolation of regions, followed by recent connections that allowed contemporary gene flow between Lake Eyre North and the Finke River (Duguid, 2011).

Surprisingly, we found limited evidence that habitat type explained the partitioning of genetic variation. There was little support for a spring-river distinction in structuring, contrary to our prediction (hypothesis 3a, Table 2). However, our prediction (3b) that springs reduce structuring remains possible if springs promote gene flow by enabling a relatively poor swimmer to maintain an enhanced spatial presence across the landscape, and thus opportunistically exploit brief dispersal windows during flooding. Indeed, a role for springs as source populations is supported by the lack of isolation-by-distance structure among locations.

Genetic diversity in springs and rivers

Flood-mediated movement between habitat types would also facilitate comparable levels of genetic diversity in springs and rivers. Due to their permanence and possible isolation, we expected springs to harbour genetic diversity by providing refuge from the bottlenecks likely in river systems (Arthington & Balcombe, 2011). However, although limited sample sizes for some springs may have affected our ability to detect differences, there was little evidence that springs contained increased or unique diversity (Fig. 3), echoing the flood-mediated gene flow also seen in shrimps and gastropods inhabiting remnant waterholes (Carini *et al.*, 2006). It is crucial, however, to consider an ecological context for water sources, in which permanent water bodies are likely to provide key refuges during drought. Thus, within a network of populations, springs are vital for maintaining diversity, as rivers are mostly ephemeral and local extinctions frequent. This creates an unavoidable sampling bias compounded by the naturally stochastic manner in which water appears and disappears in such systems, and potentially also captures individuals that have dispersed out of springs into temporary waterways. The shifts seen within localities such as Levi Creek (Fig. 4) support this scenario, whereby the rapid appearance and loss of haplotypic diversity potentially reflect temporary, stochastic connections between riverine and spring populations, although other explanations are possible. Similarly, Algebuckina Waterhole is considered its region's only permanent waterhole, yet we detected no evidence that it contained unusual levels of diversity.

The distribution of genetic variation over contemporary time

Importantly, our study derives major patterns from sampling over a range of modern time points, and thus controls for variation that can otherwise obscure phylogeographic patterns in particularly dynamic systems (Hughes *et al.*, 2013). While the sample size of some populations precludes definitive conclusions, it is clear that localized changes in haplotype frequencies occurred at individual sites (Fig. 4), highlighting that populations are subject to stochastic processes. However, at a landscape level, structuring patterns were consistent across past and current data sets (Table 2, Fig. 1), indicating that the drivers of connectivity at broad spatial scales are robust to potential, discrete effects of climatic events such as localized flooding. Whether impacts such as anthropogenic declines in spring flow rate and wetland size (Gotch, 2013) could alter the connectivity and isolation of source populations, and thus change the dynamics of the system, remains to be seen. Clearly, our data provide a valuable baseline for future assessments.

Dispersal mechanisms and connectivity

A high degree of spring-river connectivity also implies that dispersal mechanisms are efficient and allow gene flow sufficient to prevent differentiation, unlike some other cases of ecologically distinct habitats (e.g. *Daphnia* in North America, Pfrender *et al.*, 2000; poecillid fishes in Mexico, Plath *et al.*, 2010). Predominant structure that is independent of subcatchments highlights the crucial role of ephemeral surface water in shaping desert connectivity. At broader spatial scales, an unanticipated dispersal potential is underscored by the surprising lack of structure along the eastern side of Lake Eyre (Fig. 1). While sample sizes were limited, the absence of Cyt*b* sequence diversity in this part of the species' range suggests strong bottlenecks and/or colonization by a limited

number of individuals. Such homogeneity implies connectivity spanning often-dry riverine distances of over 600 km, and multiple sub-catchments, via Lake Eyre North. Conversely, the division of the Southern and Northern groups indicates that the lake's two branches are functionally isolated for *C. eremius*, despite its sometimes-surprising dispersal potential and the possible movement corridor of the Goyder Channel. An absence of gene flow through the channel could result from the rarity of simultaneous filling events of the lakes and low likelihood that gobies are sufficiently good swimmers to traverse this distance, particularly during the brief periods in which the higher elevation Goyder Channel contains water. Instead, the zones separating the two *C. eremius* genetic groups are unexpectedly short, terrestrial distances that appear to prohibit movement, including the relatively trivial 80 km separating populations on Lake Eyre's western side ('disjunction 1', Fig. 1a). The same disjunction was suggested for a wetland-based wolf spider (Gotch *et al.*, 2008), and likely results from both taxa relying on some form of surface water connectivity. In this regard, dispersal pathways that use the physiologically demanding waters of the hyper-saline Lake Eyre also highlight that physiological traits can mediate how dispersal capacity affects phylogeographic patterns (Murphy *et al.*, 2010; Eme *et al.*, 2014).

CONCLUSIONS

This study highlights the phylogeographic implications of a complex hydrological picture for aquatic populations in a hostile, arid matrix. We show that life history and resistant physiology amplifies the dispersal potential of an otherwise modest swimmer, enabling *C. eremius* to overcome large geographical distances, and potential abiotic barriers to movement. Major structuring was only seen at relatively coarse spatial scales. However, surface water connectivity is key – indeed, high temporal variance in individual populations demonstrates the action of rapid, flood-driven change – and its absence has had major consequences for population genetic structuring. Permanent spring refuges are likely to conserve genetic diversity within groups and potentially enhance gene flow by enabling fish to exploit stochastic dispersal opportunities. Such insights into the dynamics of spring-river connectivity are likely to hold increased conservation importance in future, as human-driven threats impose pressure on this remote regions' valuable water resources and unique biodiversity. Moreover, the results underscore the value of biogeographical investigations by revealing unanticipated connectivity in a small, inherently dispersal-limited species. Given the relative paucity of molecular studies of aquatic taxa in arid environments, we suggest future work will benefit from comprehensive, range-wide sampling and careful consideration of how species traits may interact with challenges to between-patch movement and persistence when conditions are extreme.

ACKNOWLEDGEMENTS

We acknowledge the traditional owners of the Kati Thanda-Lake Eyre region and particularly thank Reg Dodd and Dean Ah Chee for valuable natural and cultural insights. Thanks to Travis Gotch for helpful discussions, Michael Bertram for statistical advice, and Thor Stewart for help with the GIS. We thank Jodie and Nathan Keogh for site access, and Topi Lehtonen, Andreas Svensson, and the many contributors to the South Australian Museum tissue collection. Financial support was received from the Nature Foundation SA, the Holsworth Wildlife Endowment, and the Great Artesian Basin Coordinating Committee (to K.D.M.), and the Australian Research Council (DP120103010 to B.B.M.W.). Procedures for field sampling were approved by the Monash University Animal Ethics Committee (BSCI/2012/14). The South Australian Department of Environment and Natural Resources granted collection permits (9902391, 9902523 and 9902598).

REFERENCES

Adams, M., Wedderburn, S.D., Unmack, P.J., Hammer, M.P. & Johnson, J.B. (2011) Use of congeneric assessment to reveal the linked genetic histories of two threatened fishes in the Murray-Darling Basin, Australia. *Conservation Biology*, **25**, 767–776.

Adams, M., Raadik, T.A., Burridge, C.P. & Georges, A. (2014) Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? *Systematic Biology*, **63**, 518–533.

Alley, N.F. (1998) Cainozoic stratigraphy, palaeoenvironments and geological evolution of the Lake Eyre Basin. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **144**, 239–263.

Arthington, A.H. & Balcombe, S.R. (2011) Extreme flow variability and the 'boom and bust' ecology of fish in arid-zone floodplain rivers: a case history with implications for environmental flows, conservation and management. *Ecohydrology*, **4**, 708–720.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V.M. & Turlure, C. (2013) Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews*, **88**, 310–326.

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Bowler, J.M. & Wasson, R. (1984) Glacial age environments of inland Australia. *Late Cainozoic palaeoclimates of the southern hemisphere* (ed. by J.C. Vogel), pp. 183–208. A.A. Belkama Publishers, Rotterdam.

Byrne, M., Yeates, D., Joseph, L., Kearney, M., Bowler, J., Williams, M., Cooper, S., Donnellan, S., Keogh, J. & Leys, R. (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*, **17**, 4398–4417.

Carini, G., Hughes, J.M. & Bunn, S.E. (2006) The role of waterholes as 'refugia' in sustaining genetic diversity and variation of two freshwater species in dryland river systems (Western Queensland, Australia). *Freshwater Biology*, **51**, 1434–1446.

Chapple, D.G. & Keogh, J.S. (2004) Parallel adaptive radiations in arid and temperate Australia: molecular phylogeography and systematics of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Biological Journal of the Linnean Society*, **83**, 157–173.

Chapple, D.G., Birkett, A., Miller, K.A., Daugherty, C.H. & Gleeson, D.M. (2012) Phylogeography of the endangered Otago skink, *Oligosoma otagense*: population structure, hybridisation and genetic diversity in captive populations. *PLoS ONE*, **7**, e34599.

Cooper, S.J.B., Bradbury, J.H., Saint, K.M., Leys, R., Austin, A.D. & Humphreys, W.F. (2007) Subterranean archipelago in the Australian arid zone: mitochondrial DNA phylogeography of amphipods from central Western Australia. *Molecular Ecology*, **16**, 1533–1544.

Costelloe, J.F., Grayson, R.B., McMahon, T.A. & Argent, R.M. (2005) Spatial and temporal variability of water salinity in an ephemeral, arid-zone river, central Australia. *Hydrological Processes*, **19**, 3147–3166.

Crowley, L. & Ivantsoff, W. (1990) A review of species previously identified as *Craterocephalus eyresii* (Pisces: Atherinidae). *Proceedings of the Linnean Society of New South Wales*, **112**, 87–103.

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.

DeVogel, S.B., Magee, J.W., Manley, W.F. & Miller, G.H. (2004) A GIS-based reconstruction of late Quaternary paleohydrology: Lake Eyre, arid central Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **204**, 1–13.

Diniz-Filho, J.A.F., Soares, T.N., Lima, J.S., Dobrovolski, R., Landeiro, V.L., de Campos Telles, M.P., Rangel, T.F. & Bini, L.M. (2013) Mantel test in population genetics. *Genetics and Molecular Biology*, **36**, 475–485.

Duguid, A. (2011) *Wetlands of the Great Artesian Basin Water Control District (Northern Territory)*. Northern Territory Government Department of Natural Resources, Environment, the Arts and Sport, Alice Springs.

Duvernell, D.D. & Turner, B.J. (1999) Variation and divergence of Death Valley pupfish populations at retrotransposon-defined loci. *Molecular Biology and Evolution*, **16**, 363.

Eme, D., Malard, F., Colson-Proch, C., Jean, P., Calvignac, S., Konecny-Dupre, L., Hervant, F. & Douady, C.J. (2014) Integrating phylogeography, physiology and habitat modelling to explore species range determinants. *Journal of Biogeography*, **41**, 687–699.

Faulks, L.K., Gilligan, D.M. & Beheregaray, L.B. (2010) Islands of water in a sea of dry land: hydrological regime predicts genetic diversity and dispersal in a widespread fish from Australia's arid zone, the golden perch (*Macquaria ambigua*). *Molecular Ecology*, **19**, 4723–4737.

Fensham, R., Silcock, J., Kerezsy, A. & Ponder, W. (2011) Four desert waters: setting arid zone wetland conservation priorities through understanding patterns of endemism. *Biological Conservation*, **144**, 2459–2467.

Fu, Y.-X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Fujioka, T. & Chappell, J. (2010) History of Australian aridity: chronology in the evolution of arid landscapes. *Geological Society, London, Special Publications*, **346**, 121–139.

Glover, C. (1971) *The taxonomy and biology of* Chlamydogobius eremius *(Zietz, 1896)*. From the Department of Zoology, University of Adelaide.

Glover, C. (1982) Adaptations of fishes in arid Australia. *Evolution of the flora and fauna of arid Australia* (ed. by W.R. Barker and P.J.M. Greenslade), pp. 241–246. Peacock Publications, Adelaide.

Gomon, M.F. & Bray, D.J. (2011) Desert Goby, *Chlamydogobius eremius* (Zietz 1896). *Fishes of Australia*. Available at: http://www.fishesofaustralia.net.au/home/species/93 (accessed 10 December 2014).

Google Inc. (2011) Google Earth (Version 6.0.3.2197) [Computer software]. Available at: http://www.google.com/earth/(accessed 12 February 2012).

Gotch, T.B. (ed.) (2013) *Allocating water and maintaining springs in the Great Artesian Basin. Volume V: Groundwater-dependent ecosystems of the western Great Artesian Basin*. National Water Commission, Canberra.

Gotch, T.B., Adams, M., Murphy, N.P. & Austin, A.D. (2008) A molecular systematic overview of wolf spiders associated with Great Artesian Basin springs in South Australia: evolutionary affinities and an assessment of metapopulation structure in two species. *Invertebrate Systematics*, **22**, 151–165.

Habeck-Fardy, A. & Nanson, G.C. (2014) Environmental character and history of the Lake Eyre Basin, one-seventh of the Australian continent. *Earth-Science Reviews*, **132**, 39–66.

Hammer, M.P., Adams, M., Unmack, P.J. & Walker, K.F. (2007) A rethink on *Retropinna:* conservation implications of new taxa and significant genetic sub-structure in Australian smelts (Pisces: Retropinnidae). *Marine and Freshwater Research*, **58**, 327–341.

Hammer, M.P., Adams, M. & Hughes, J.M. (2013) Evolutionary processes and biodiversity. *Ecology of Australian freshwater fishes* (ed. by P. Humphries and K. Walker), pp. 49–79. CSIRO Publishing, Collingwood.

Hughes, J.M., Huey, J.A. & Schmidt, D.J. (2013) Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? *Freshwater Biology*, **58**, 951–966.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S. & Duran, C. (2012) Geneious Basic: an integrated and extend-

able desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.

Kotwicki, V. (1986) *Floods of Lake Eyre*. Engineering and Water Supply Department, Adelaide.

Larson, H.K. (1995) A review of the Australian endemic gobiid fish genus *Chlamydogobius*, with description of five new species. *The Beagle: Records of the Museums and Art Galleries of the Northern Territory*, **12**, 19–51.

Legendre, P. & Legendre, L. (2012) *Numerical ecology*. Elsevier, Amsterdam.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Magee, J.W., Miller, G.H., Spooner, N.A. & Questiaux, D. (2004) Continuous 150 ky monsoon record from Lake Eyre, Australia: insolation-forcing implications and unexpected Holocene failure. *Geology*, **32**, 885–888.

McGlashan, D.J. & Hughes, J.M. (2001) Low levels of genetic differentiation among populations of the freshwater fish *Hypseleotris compressa* (Gobiidae: Eleotridinae): implications for its biology, population connectivity and history. *Heredity*, **86**, 222–233.

McMahon, T.A., Murphy, R., Little, P., Costelloe, J.F., Peel, M.C., Chiew, F.H.S., Hayes, S., Nathan, R. & Kandel, D.D. (2005) *Hydrology of the Lake Eyre Basin*. Sinclair Knight Merz, Armadale.

McNeil, D. & Schmarr, D. (2009) Recovery of Lake Eyre Basin fishes following drought: 2008/2009 fish survey report. SARDI Publication No. F2009/000407-1. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.

Meffe, G.K. & Vrijenhoek, R.C. (1988) Conservation genetics in the management of desert fishes. *Conservation Biology*, **2**, 157–169.

Murphy, N.P., Guzik, M.T. & Wilmer, J.W. (2010) The influence of landscape on population structure of four invertebrates in groundwater springs. *Freshwater Biology*, **55**, 2499–2509.

Murphy, N.P., Breed, M.F., Guzik, M.T., Cooper, S.J.B. & Austin, A.D. (2012) Trapped in desert springs: phylogeography of Australian desert spring snails. *Journal of Biogeography*, **39**, 1573–1582.

Papadopoulou, A., Anastasiou, I., Keskin, B. & Vogler, A.P. (2009) Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. *Molecular Ecology*, **18**, 2503–2517.

Pfrender, M.E., Spitze, K. & Lehman, N. (2000) Multi-locus genetic evidence for rapid ecologically based speciation in *Daphnia*. *Molecular Ecology*, **9**, 1717–1735.

Pickup, G., Allan, G. & Baker, V. (1988) History, palaeochannels and palaeofloods of the Finke River, central Australia. *Fluvial geomorphology of Australia* (ed. by R.F. Warner), pp. 177–200. Academic Press, Sydney.

Plath, M., Hermann, B., Schroder, C., Riesch, R., Tobler, M., de Leon, F.J.G., Schlupp, I. & Tiedemann, R. (2010) Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. *BMC Evolutionary Biology*, **10**, 256.

Ponder, W.F. (1986) Mound Springs of the Great Artesian Basin. *Limnology in Australia* (ed. by P. De Deckker and W.D. Williams), pp. 403–420. Springer, Netherlands.

Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Richardson, B.J., Baverstock, P.R. & Adams, M. (1986) *Allozyme electrophoresis: a handbook for animal systematics and population studies*, 1st edn. Academic Press, Sydney.

Richardson, J.L. (2012) Divergent landscape effects on population connectivity in two co-occurring amphibian species. *Molecular Ecology*, **21**, 4437–4451.

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.

Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Sharma, S. & Hughes, J. (2011) Genetic structure and phylogeography of two freshwater fishes, *Rhadinocentrus ornatus* and *Hypseleotris compressa*, in southern Queensland, Australia, inferred from allozymes and mitochondrial DNA. *Journal of Fish Biology*, **78**, 57–77.

Systat Software, Inc. (2013) SYSTAT (version 13) [Computer software]. Available at: http://www.systat.com/(accessed 4 December 2013).

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Thompson, G.G. & Withers, P.C. (2002) Aerial and aquatic respiration of the Australian desert goby, *Chlamydogobius eremius*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **131**, 871–879.

Unmack, P.J. (2013) Biogeography. *Ecology of Australian freshwater fishes* (ed. by P. Humphries and K. Walker), pp. 25–48. CSIRO Publishing, Collingwood.

Unmack, P.J., Hammer, M.P., Adams, M., Johnson, J.B. & Dowling, T.E. (2013) The role of continental shelf width in determining freshwater phylogeographic patterns in south-eastern Australian pygmy perches (Teleostei: Percichthyidae). *Molecular Ecology*, **22**, 1683–1699.

Wager, R. & Unmack, P.J. (2000) *Fishes of the Lake Eyre catchment of central Australia*. Queensland Department of Primary Industries.

Yang, X., Xue, D. & Han, H. (2013) The complete mitochondrial genome of *Biston panterinaria* (Lepidoptera: Geometridae), with phylogenetic utility of mitochondrial genome in the Lepidoptera. *Gene*, **515**, 349–358.

Young, E.F., Belchier, M., Hauser, L., Horsburgh, G.J., Meredith, M.P., Murphy, E.J., Pascoal, S., Rock, J., Tysklind, N. & Carvalho, G.R. (2015) Oceanography and life

history predict contrasting genetic population structure in two Antarctic fish species. *Evolutionary Applications*, **8**, 486–509.

SUPPORTING INFORMATION

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

Appendix S1 Extended locality and sample information for *Chlamydogobius* samples.
Appendix S2 Additional characterization of genetic variation in *Chlamydogobius* sample sets.

BIOSKETCH

This paper forms part of **Krystina Mossop's** PhD research on the molecular and behavioural ecology of the desert goby, supervised by **Bob Wong** and **David Chapple**. She is particularly interested in the issues underlying connectivity and persistence of aquatic species in changing environments. Shared research interests among the authors include biogeography, population genetic structuring, phylogeography and behavioural ecology.

Author contributions: K.D.M., M.A., P.J.U. and D.G.C. conceived the ideas and obtained funding for the project, K.D.M., K.L.S.D. and M.A. collected and analysed the data, K.D.M., M.A., D.G.C. and B.B.M.W. wrote the original manuscript, which was then edited by all authors.

Editor: Mark Carine