A genome-wide search for local adaptation in a terrestrial-breeding frog reveals vulnerability to climate change

Running title: Genetic diversity and local adaptation in frogs

Deanne Cummins[1]
W. Jason Kennington[1]
Tabitha Rudin-Bitterli[1]
Nicola J. Mitchell[1]

[1]School of Biological Sciences, The University of Western Australia, 35 Stirling Highway Crawley WA 6005, Australia

Correspondence:
Deanne Cummins
School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia

Email: deanne.cummins@research.uwa.edu.au

Paper type: Primary research

Abstract

Terrestrial-breeding amphibians are likely to be vulnerable to warming and drying climates, as their embryos require consistent moisture for successful development. Adaptation to environmental change will depend on sufficient genetic variation existing within or between connected populations. Here we use single nucleotide polymorphism (SNP) data to investigate genome-wide patterns in genetic diversity, gene flow and local adaptation in a terrestrial-breeding frog (*Pseudophryne guentheri)* subject to a rapidly drying climate and recent habitat fragmentation. The species was sampled across twelve central and range-edge populations (192 samples), and strong genetic structure was apparent, as were high inbreeding coefficients. Populations showed differences in genetic diversity, and one population lost significant genetic diversity in a decade. More than 500 SNP loci were putatively under directional selection, and 413 of these loci were correlated with environmental variables such as temperature, rainfall, evaporation and soil moisture. One locus showed homology to a gene involved in the activation of maturation in *Xenopus* oocytes, which may facilitate rapid development of embryos in drier climates. The low genetic diversity, strong population structuring and presence of local adaptation revealed in this study shows why management strategies such as assisted gene flow may be necessary to assist isolated populations to adapt to future climates.

Keywords

Genome-wide, genetic diversity, local adaptation, SNP, climate change, amphibian, *Pseudophryne guentheri*

Introduction

An alarming number of amphibian species, approximately a third worldwide, have declined or become extinct since 1970 (Lips, Diffendorfer, Mendelson, & Sears, 2008; Perl et al., 2017; Pounds, Fogden, & Campbell, 1999; Van Rooij, Martel, Haesebrouck, & Pasmans, 2015). This global decline has been attributed to factors such as habitat fragmentation, disease, climate change, increased UV-B radiation and introduced species (Beebee & Griffiths, 2005; Collins & Storfer, 2003; Lips et al., 2008; Van Rooij et al., 2015; Wake, 2012). Climate change is an indirect contributor to amphibian population declines by compounding the effects of other threats (Pounds et al., 1999; Winter et al., 2016) but also directly impacts amphibian populations by causing physiological tolerances to be breached, and by reducing the rate and scale of dispersal (Lawler, Shafer, Bancroft, & Blaustein, 2010).

Amphibian populations can potentially respond to new selection pressures via evasion, phenotypic plasticity or genetic adaptation (Urban, Richardson, & Freidenfelds, 2014). However, evasion of unfavourable climates is not an option for species whose dispersal is restricted due to habitat fragmentation (Beebee, 1995; Lawler et al., 2010; Smith & Green, 2005). Similarly, phenotypic plasticity will not necessarily allow persistence under continued directional environmental change, as there are limits to the extent of non-genetic responses (Auld, Agrawal, & Relyea, 2010; Dewitt, Sih, & Wilson, 1998; Gienapp, Teplitsky, Alho, Mills, & Merila, 2008). For many species, genetic adaptation represents the best option for population resilience in the face of continued directional change in their environments (Sgrò, Lowe, & Hoffmann, 2011).

The capacity of populations to adapt to new selection pressures will depend on their ability to evolve phenotypes that suit their new environments (Tigano & Friesen, 2016). This ability in turn depends on the level of standing genetic variation within a population upon which natural selection can act (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). Short-lived species can evolve rapidly in response to climate change if they possess sufficient genetic variation in tolerance traits (Sgrò et al., 2011), and higher levels of genetic diversity provide a greater probability of achieving

allelic combinations that confer beneficial phenotypes (Barrett & Schluter, 2008). In the absence of genetic variation, there is now strong evidence for an increased risk of extinction in wild populations (Spielman, Brook, & Frankham, 2004). Further, restricted gene flow between populations subject to different environmental pressures will increase their genetic divergence, and result in local adaptation (Slatkin, 1987).

For many amphibian species, environmental disturbances will be experienced as increasing temperatures and decreasing rainfall (Bernhardt & Leslie, 2013). An overall decrease in rainfall, plus increasing variation in precipitation, such as the incidence and severity of drought events, will likely have a severe negative effect on both aquatic and terrestrial-breeding anurans as breeding success, phenology and migration are tightly associated with the presence of water (Todd & Winne, 2006; Walls, Barichivich, & Brown, 2013). Terrestrial-breeding frogs are especially vulnerable to a reduction in rainfall, as seasonal rainfall causes increases in soil moisture and the appearance of standing water that are critical to breeding success. For example, frogs in the Australian *Pseudophryne* genus lay their eggs in burrows in direct contact with the soil, and rainfall keeps embryos hydrated and ultimately floods the burrows (Eads, Mitchell, & Evans, 2012). Burrow flooding initiates hatching and provides standing water for the completion of metamorphosis (Bradford & Seymour, 1988). Climate change is apparent across the continent-wide distribution of this genus, but is particularly marked in the southwest of Australia. This bioregion has experienced substantial declines in autumn rainfall over the past 40 years, and climate projections suggest that temperature and evaporation will increase, while rainfall and soil water availability will further decrease (Bates, Hope, Ryan, Smith, & Charles, 2008). The frequency of years with exceptionally low soil moisture is predicted to increase to approximately once every six years by 2030, with a possible 60% decrease in autumn rainfall by 2070 under the most extreme climate change scenario (Bates *et al.* 2008).

In this study we conducted a genome-wide analyses of genetic variation in the crawling frog, *Pseudophryne guentheri* — one of 17 terrestrial-breeding amphibian

117 species endemic to the southwest of Australia. The species is an ideal model for
118 studying responses to climate change due to marked intraspecific variation in the
119 ability of embryonic and adult life stages to withstand desiccation (Rudin-Bitterli,
120 Evans, & Mitchell, 2018). Based on a quantitative genetics study of a single
121 population of this species, Eads *et al.* (2012) suggested it has limited capacity to
122 adapt to a drying climate, due to low levels of heritable genetic variation in traits
123 associated with desiccation tolerance. Here, we expand to a multi-population study
124 and use a genotype-by-sequencing method to generate genome-wide single
125 nucleotide polymorphism (SNP) markers to assess population genetic structure and
126 test for signatures of local adaptation to climatic variables. Taken together, these
127 data provide a means for assessing the susceptibility of this species to future climate
128 change.
129
130 **Materials and Methods**
131
132 **Sample selection**
133 Tissue samples from 192 individual *P. guentheri* collected from 12 geographically
134 distinct populations were selected for genetic analysis. The populations were
135 distributed across approximately half the known range of *P. guentheri* in
136 southwestern Australia, and spanned environmental gradients including average
137 annual temperature, which decreases from north to south, and average annual
138 precipitation, which increases from north to south, and is higher in more coastal
139 localities (Fig. 1).
140
141 Tissue samples were obtained from the Western Australian Museum and collections
142 of the authors (NM, TBR), and included the muscle or liver of adult frogs (mostly
143 males) or the tail of a tadpole (sex unknown). Samples from most locations (Binnu,
144 Chidlow, Dudinin, Flint plot, Mullewa, Ridgefield farm and Yalgoo) were collected
145 during the 2016 autumn-winter breeding season. For two sites, samples were
146 collected in two different years (Flint plot: 2006 and 2016, Pinjar: 2007 and 2008),
147 which allowed analysis of temporal variation. Given the short period between the
148 temporal samples from Pinjar, they are unlikely to represent different generations, but

were nonetheless included to have independent samples of the same population and test for genetic changes over one year. Of the remaining populations, two were sampled in 1992 (Dalwallinu and Wyalkatchem) and Spalding Park was sampled in 1993. In addition to samples of the target species (*P. guentheri*), tissues samples of a sympatric species (*Pseudophryne occidentalis;* Fig. 2a) collected 9 km east of Yalgoo in 2009 were included. This population occurred only 10 km from the Yalgoo *P. guentheri* population and so was used to test for potential hybridization, as admixture between lineages can occur at species boundaries within this taxonomic group (O'Brien, Keogh, Silla, & Byrne, 2018).

SNP genotyping and screening

Tissue samples were sent to Diversity Arrays Technology to generate a genome-wide single nucleotide polymorphism (SNP) data set, using a genotype-by-sequencing approach (DArT-seq; http://www.diversityarrays.com/). DArTseq involves a combination of DArT complexity reduction methods and Illumina sequencing and is similar to restriction site-associated DNA sequencing (RADseq; Davey et al., 2010). DArTseq has three major advantages including a lower DNA input, greater tolerance to low quality DNA and a higher call rate (Sánchez-Sevilla et al., 2015). Four enzyme systems for complexity reduction were tested and *PstI-SphI* was chosen. The *PstI*-compatible adapter comprised an Illumina flow cell attachment sequence, a sequencing primer and a staggered barcode region of varying lengths. The reverse adapter (*Sph*I-compatible) contained the Illumina flow cell attachment sequence and an *SphI* overhang sequence. DNA samples were processed in digestion/ligation reactions (Kilian et al., 2012). Only ligated fragments with both a *PstI* and *SphI* adapter were amplified by PCR with an initial denaturation step at 94 °C for 1 minute, followed by 30 cycles with a temperature profile as follows: denaturation at 94 °C for 20 seconds, annealing at 58 °C for 30 seconds and extension at 72 °C for 45 seconds, with an additional final extension (Melville et al., 2017).

Following PCR amplification, the products from each sample were pooled and applied to a cBot (Illumina) bridge PCR and then sequenced on an Illumina Hiseq2500. Single read sequencing was run for 77 cycles (Kilian et al., 2012).

Sequences were processed using propriety DArTseq analytical pipelines. The primary pipeline was used to filter poor quality sequences from FASTQ files (Kilian *et al.* 2012). Identical sequences were collapsed into "fastqcoll files" and data was groomed using DArT's propriety algorithm to correct for low quality bases from singleton reads, using collapsed reads with multiple members as a template. Groomed data was used in the secondary pipeline for SNP calling (Kilian et al., 2012).

Following the generation of 103 608 loci, DArT loci that were genotyped in fewer than 85% of samples, had a minor allele frequency (MAF) less than 0.05, or low sequence coverage (<5), were removed from the dataset prior to analysis. Then, to screen for cryptic lineages and possible hybrids between *P. guentheri* and *P. occidentalis,* a neighbour joining tree based on Nei's genetic distance (Nei, 1972) was constructed using the R package POPPR version 2.5.0 (Kamvar, Tabima , & Grünwald, 2014).

Population structure and genetic diversity

Loci were divided into two groups: neutral loci and loci that are potentially under directional or balancing selection. Loci potentially under selection were identified using the *fdist2* method in LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008). This method measures population divergence by generating a global neutral distribution for F_{ST}, under Wright's Island model (Charlesworth, 1998; Wright, 1931) and estimates the expected heterozygosity and unbiased F_{ST} values for each locus. It is considered a conservative method of identifying neutral loci as retention of loci under weak selection is minimised, which could bias results (Thomas, Kennington, Evans, Kendrick, & Stat, 2017). Analyses were conducted assuming an infinite alleles mutation model, based on 500 000 simulations, and using neutral mean F_{ST}, a 95% confidence interval and a false discovery rate of 0.1. Loci with a probability between 0.05 and 0.95 were retained as neutral loci, as they were not considered significantly different from the neutral mean F_{ST}. Neutral loci were screened for conformity to Hardy-Weinberg equilibrium (HWE) using GENODIVE 2.0b.27 and the Benjamini & Yekutieli (BY) method was used to correct for multiple comparisons

(Benjamini & Yekutieli, 2001). Loci that deviated consistently from HWE (in more than three populations) were removed.

Only putatively neutral loci were used for the following analyses. Population structure was assessed by calculating Weir & Cockerham's F_{ST} using the R package 'hierefstat' (Goudet, 2005). The significance of F_{ST} values was determined by bootstrapping 100 replicates (Weir & Cockerham, 1984) and we corrected for multiple comparisons using the BY method (Benjamini & Yekutieli, 2001). Population structure was also assessed by performing a discriminant analysis of principle components (DAPC) using the R package 'adegenet' (Jombart, Devillard, & Balloux, 2010). No *a priori* information about sampling location were used in the analysis. The optimal number of principle components and discriminant functions were retained using a cross-validation method (Krzanowski, 1987).

The R package 'hierefstat' was also used to calculate inbreeding coefficients (F_{IS}) and expected heterozygosity (H). Bootstrapping based on 100 replicates was performed over loci to generate confidence intervals, to assess the significance of F_{IS} values for each population and the BY method was used to correct for multiple comparisons (Benjamini & Yekutieli, 2001). A Friedman rank sum test was used to determine whether genetic variation differed significantly among populations, and a Wilcoxon rank sum test was used to determine which populations were differed significantly from each other (Bauer, 1972; Friedman, 1937), again, correcting for multiple comparisons using the BY method (Benjamini & Yekutieli, 2001). The R package 'snpReady' was used to calculate the estimated effective population size (N_e) for each population, using a single sample method based on heterozygote excess (Robertson, 1965).

Relationships between measures of genetic diversity and the F_{IS} of each population against five environmental variables were tested using regression analysis. Environmental variables examined included monthly total rainfall, minimum and maximum temperature, average evaporation and monthly soil moisture (upper layer). All environmental variables were obtained from the Bureau of Meteorology (http://www.bom.gov.au/) for the specific coordinates of each population, which were

interpolated from weather station data. Monthly values were averaged across 38 years (1980-2017) for all environmental variables.

We also conducted a Bayesian clustering approach with the software package STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) and using sNMF analysis (Frichot, Mathieu, Trouillon, Bouchard, & François, 2014) to check for consistent results. The number of genetic clusters (K) tested, ranged from one to 12 with ten replicates per K and no prior information about sampling location. A burn-in of 10 000 was followed by 100 000 MCMC replicates, assuming correlated allele frequencies. STRUCTURE HARVESTER (Earl & vonHoldt, 2012) was used to determine the optimal number of populations following the delta K approach (Evanno, Regnaut, & Goudet, 2005). The sNMF analysis was conducted using the R package 'lea' (Frichot & François, 2015) with similar settings for K to support the STRUCTURE results and represent them graphically, using a method that is robust to departures from traditional population genetic model assumptions including HWE (Frichot et al., 2014). Finally, a mantel test was conducted using the R package 'ade4' to test the correlation between genetic (using Provesti distance; Alonso, 1975; Prevosti, 1974) and geographic pairwise distance matrices.

Signatures of local adaptation

To identify loci putatively under directional selection, a Bayesian model-based approach was conducted using BAYESCAN 2.0, using default settings (Foll & Gaggiotti, 2008). This method has been shown to have lower rates of type 1 errors than the *fdist2* method under a hierarchal model in ARLEQUIN (Narum & Hess, 2011). BAYESCAN was also conducted using just the two samples collected from Flint Plot to determine if any temporal variation at this location could be attributed to directional environmental selection. Loci with a q-value of less than 0.05 were considered to be under direction selection.

One limitation of the BAYESCAN approach for detecting loci under directional selection ('outlier loci') is the relatively high false discovery rate (40%; Luu, Bazin, & Blum, 2017). To filter the resulting loci for false positive results a second outlier detection method PCADAPT was used, which has a lower false discovery rate (10%;

Luu et al., 2017). A second limitation of the BAYESCAN approach that it cannot determine which environmental variables may be driving selection (Villemereuil & Gaggiotti, 2015). Hence, to infer the particular environmental variable underlying directional selection on loci, the software package BAYESCENV was used (with default settings) to test whether F_{ST} increased with environmental differentiation (Villemereuil & Gaggiotti, 2015). As outlined earlier, the environmental variables examined were monthly total rainfall, minimum and maximum temperature, average evaporation and monthly soil moisture. Each variable was standardized to represent an "environmental distance" between the value observed at each population and the reference value (average across all populations) for each variable, as per developers instructions (Villemereuil & Gaggiotti, 2015).

To identify if any loci under directional selection (as identified by all three approaches) were in genic regions that coded for proteins, a general nucleotide blast search ('blastn') was conducted, followed by specifically blasting reference nucleotide sequences against four available frog genomes: the African clawed frog (*Xenopus laevis*), the Western clawed frog (*Xenopus tropicalis*) the Tibetan frog (*Nanorana parkeri*), and the cane toad (*Rhinella marina*) (available at: www.ncbi.nlm.nih.gov/genome/). To ensure only highly similar sequences were used for investigation of outlier SNP function, a BLAST match was considered significantly similar if it had greater than 80% coverage, greater than 90% identity and an E-score of less than 1×10^{-10} (Pearson, 2013).

Results

SNP genotyping and screening

Of the 103,608 high quality SNPs produced by the DArT propriety pipeline, 12,787 (12.3%) met our selection criteria. A neighbor-joining tree generated by this subset of SNPs showed that the Yalgoo *P. guentheri* were genetically distinct and were most likely a cryptic lineage (Fig. 2b). Hence to allow examination of intraspecific variation within *P. guentheri*, all Yalgoo sequences and the *P. occidentalis* sequences were excluded from further analysis.

Population structure and genetic diversity

Analyses of the neutral loci indicated high levels of genetic differentiation among populations. Pairwise F_{ST} estimates between sites ranged from 0.052 to 0.327, with most pairwise F_{ST} values significantly different to zero (Table S4). Strong population structure was also evident from the inference of individual ancestry coefficients and DAPC (Fig. 2c and Fig. 2d, respectively). The DAPC grouped individuals from each population sample into a distinct genetic cluster. A clustering analysis using the R package 'lea' was consistent with the results of a STRUCTURE analysis, showing $K = 5$ to be the most likely number of ancestral populations.

A plot of individual ancestry coefficients, based on $K = 5$, grouped individuals from the northern populations of Binnu, Mullewa and Spalding Park in a single cluster. Individuals from central regions (Wyalkatchem and Dalwallinu) were placed in a separate cluster, but showed some admixture with the cluster representing the northern populations. Pinjar samples collected in 2007 and 2008 clustered together and most of the southern populations formed distinct genetic clusters, showing very small levels of admixture. Strong geographic structure was also evident from the significant positive relationship between pairwise F_{ST} and geographical distance (Fig. 2e).

Genetic variation within populations was relatively uniform (expected heterozygosity ranging from 0.193 to 0.247; Fig. 3), although there were several significant differences between populations, and between samples taken from the same population in different years. In general, populations with greater geographical separation and sampled more than a year apart were more likely to be significantly different (Fig. 2e & Fig. 3). Temporal variation was evident in the Flint Plot population, where expected heterozygosity was significantly lower in the more recent 2016 sample (Fig. 3 & Table S3). All populations showed relatively high, significant median F_{IS} values, ranging from 0.161 to 0.318 and low estimates of N_e ranging from 18.2 to 51.9 (Table S1). The lowest N_e were found at Ridgefield Farm, Wyalkatchem and Flint Plot with the Flint Plot population showing a slight decline in N_e over ten years (Table S1).

Genetic variation (expected heterozygosity) and F_{IS} showed significant linear relationships with evaporation, maximum temperature and latitude, but not with minimum temperature, rainfall or soil moisture (Fig. 4; Table S2). Lower latitude populations and those with higher maximum temperatures and higher levels of evaporation had less genetic variation and lower inbreeding coefficients than did higher latitude populations characterized by lower maximum temperatures and evaporation.

Signatures of local adaptation

When all populations were considered, BAYESCAN identified 1590 outlier loci that were putatively under directional selection. PCADAPT identified 783 outlier loci, with 560 loci identified by both approaches. The BAYESCENV analysis on these same populations identified 413 unique loci correlated with environmental variables (Fig. S2), 308 were correlated with soil moisture, 260 were correlated with rainfall, 182 were correlated with minimum temperature, 181 were correlated with maximum temperature and 171 were correlated with evaporation.

Using loci identified by all three outlier analyses as putatively under directional selection, 413 BLAST searches were conducted, and two blast results matched the selection criteria. One locus that showed strong correlation with evaporation and soil moisture had a high sequence identity and coverage with multiple transcript variants of Protein Kinase C, zeta mRNA of two species of clawed frogs, *Xenopus tropicalis* and *Xenopus laevis* (Table 1). Another locus showing strong correlation with all environmental variables had 100% coverage and 93% identity with a region upstream of chromatin modifying protein (Chmp2a) in Atlantic salmon (*Salmo salar*).

Discussion

Given the projections of a drying climate in the southwest of Australia, information on the partitioning of genetic variation within vulnerable species is critical for predicting their capacity to adapt genetically. In this study of *P. guentheri*, we identify six to seven distinct genetic clusters that potentially exhibit high levels of inbreeding and

signatures of local adaptation. Populations at the range margins had amongst the lowest levels of genetic variation, and one population sampled a decade apart showed declining genetic variation over time. Recent habitat fragmentation in southwestern Australia has constrained gene flow in many species. Restricted gene flow, coupled with the low genetic diversity as we reveal here, suggests that *P. guentheri* has a limited capacity to adapt to rapid climate change.

Population structure and genetic variation

Strong population genetic structure can reflect naturally low dispersal capability and high site fidelity, as known in other terrestrial-breeding frogs (Driscoll, 1997, 1998; Smith & Green, 2005). Terrestrial-breeding frogs are restricted to breeding sites that are consistently moist, and/or will reliably flood (e.g. near ephemeral creek lines), often leading to isolation across different drainages. Some species that occupy forested drainage lines (e.g. *Geocrinia alba* and *G. vitellina*) show extreme site philopatry, and exchange of males between adjacent populations is either rare or does not occur at all (Driscoll, 1997). Fidelity to breeding areas, and even to specific nest sites is also known in the *Pseudophryne* genus (Heap, Stuart-Fox, & Byrne, 2015; Mitchell, 2001, 2005) and could in part explain the strong genetic structure in *P. guentheri*. In addition, *P. guentheri* tadpoles are restricted to small, temporary pools and are unlikely to get washed between populations (Bradford & Seymour, 1988), hence low dispersal in the larval phase could also contribute to the strong population structure evident in this study.

Habitat fragmentation is also well known to influence species' population structure (Andersen, Fog, & Damgaard, 2004; Burns, Eldridge, & Houlden, 2004; Dixo, Metzger, Morgante, & Zamudio, 2009; Levy, Kennington, Tomkins, & Lebas, 2010; Richter, 2009; Spear & Storfer, 2008). Most of our study populations occur in the Western Australian Wheatbelt, a once-forested area that has been extensively cleared since the early 1900s for agriculture with less than ten percent of native vegetation remaining in small isolated remnants (Hobbs, 1993; Jarvis, 1986; Saunders, 1989). Other study populations outside the Wheatbelt region, such as Pinjar, occur adjacent to land that has been cleared for agriculture or for the expansion of the city of Perth (Weller, 2009). Land clearing for agriculture has been

found to influence the abundance and composition of amphibian populations (Gray, Smith, & Leyva, 2004) and inhibit genetic exchange between them, causing genetic differentiation (Lenhardt, Brühl, Leeb, & Theissinger, 2017). Specifically in the Wheatbelt region, land clearing has been shown to reduce gene flow between populations of the granite outcrop-dwelling lizard *Ctenophorus ornatus*, with populations occupying outcrops on agricultural land showing lower genetic variation and greater genetic divergence relative to populations in a nature reserve (Levy et al., 2010). While the dispersal ability of *P. guentheri* is unknown, land clearing almost certainly creates a barrier to gene flow in this species due to the risk of desiccation when crossing the agricultural matrices between breeding sites.

While substantial population structure suggests limited gene flow between populations, the strong geographical patterns (including isolation-by-distance) suggest that *P. guentheri* populations have not been isolated in the past. Northern populations were clustered together in the plot of ancestry coefficients, which could be the result of historic range expansion in a northerly direction or because they have been isolated by habitat fragmentation more recently than the southern populations. When range expansion occurs via several founder effects, genetic drift will effectively be accelerated (Slatkin & Excoffier, 2012), resulting in relatively lower levels of genetic diversity in the northern populations and a higher genetic similarity between them, as found in this study. Notably, many studies have documented a temporal lag in changes to genetic population structure in response to habitat fragmentation, especially in less mobile organisms (Burel et al., 1998; Holzhauer, Ekschmitt, Sander, Dauber, & Wolters, 2006; Landguth et al., 2010; Levy, Tomkins, Lebas, & Kennington, 2013; Spear & Storfer, 2008). Hence, the timing of land clearing may have some explanatory power in our study. Based on an account of clearing patterns in the Wheatbelt (Jarvis, 1986), fragmentation around the three northern (Binnu, Mullewa and Spalding Park) and the two central populations (Dalwallinu and Wyalkatchem) occurred in 1890, 40 years after clearing around the southern populations (Flint plot, Ridgefield farm, Dudinin). More recent land clearing around the northern and central populations could explain their stronger genetic clustering,

433 although with a time difference of 40 years, this pattern could also be due to historic
434 range expansion.

435 **Isolation and inbreeding**

436 The significantly positive F_{IS} values detected in this study are consistent with limited
437 gene flow between populations. In addition, small N_e have likely given rise to high
438 levels of genetic drift (Ellstrand & Elam, 1993; Slatkin, 1987). The significant decline
439 in genetic diversity within the Flint plot population over ten years (Fig. 3; Table S1)
440 provides some evidence that gene flow has been insufficient to mitigate the negative
441 effects of genetic drift. If populations remain isolated, further declines in genetic
442 diversity would be expected. This makes populations vulnerable if they lack sufficient
443 genetic variation to adapt to changing environments or to evolve resistance to
444 introduced diseases (Collins & Storfer, 2003; Wake & Vredenburg, 2008).
445 Furthermore, low rainfall has a negative impact on the breeding success of species in
446 this genus (e.g. *P. bibronii*, Mitchell, 2001). Flint plot in 2006 had very low autumn
447 and winter rainfall (Fig. S1), very few males mated and clutch failure was common
448 (N. J. Mitchell, unpublished data). This process likely repeated in 2010 when rainfall
449 was also very low (Fig. S1), but no visits to the site were made in 2010 to confirm this
450 pattern. Recurring recruitment failure due to years of very low rainfall may have
451 compounded the effects of genetic drift in the small, isolated Flint Plot population,
452 further reducing genetic diversity. Future studies should include temporal analysis of
453 genetic variation in multiple *P. guentheri* populations to establish if the decreasing
454 diversity is widespread, and to identify the factors associated with significant
455 declines.
456
457 In general, high F_{IS} values suggest high levels of inbreeding within populations.
458 Breeding between related individuals can lead to reductions in fitness, including
459 lower mating success, reduced fecundity, increased sterility, slower development and
460 increased susceptibility to environmental stress (Bijlsma, Bundgaard, & Van Putten,
461 1999; Frankham, 1995; Roff, 1998). In small populations with an N_e less than 100 (as
462 estimated for all of the population in this study), inbreeding can lead to mutational
463 meltdown (Keller & Waller, 2002). The F_{IS} values of *P. guentheri* populations are
464 comparable to some of the highest F_{IS} values in populations of the European tree

frog *Hyla arborea*, which showed signs of inbreeding depression on larval survival (Andersen et al., 2004). In some cases, natural selection can 'purge' deleterious alleles from the population through strong selection against them (Reed, Lowe, Briscoe, & Frankham, 2003), however, the extent of purging depends on many factors and is often inefficient especially in small populations where selection is less effective and genetic drift dominates (Keller & Waller, 2002). In addition, purging is environmentally-dependent, as changing or deteriorating environments can evoke previously 'concealed' genetic load to become expressed, resulting in increased inbreeding depression in harsher environments (Bijlsma et al., 1999). Hence high inbreeding coefficients in a changing climate are likely disadvantageous. While it is possible in this study that inbreeding coefficient values were inflated due to the SNP markers used, similar F_{IS} values were obtained even when loci that consistently deviated from HWE were removed.

Intriguingly, F_{IS} values and genetic diversity both decreased significantly with increasing temperature and evaporation (Fig. 4). Lower genetic diversity near the northern range edge could reflect more frequent drought years that negatively affect reproductive success and keep populations small (Walls et al., 2013). Alternatively, lower genetic diversity may simply reflect less migration to range margins than occurs at the center of a species range (the central-marginal hypothesis; (Eckert, Samis, & Lougheed, 2008). On this basis, higher levels of inbreeding would be expected in our populations near the northern range edge, yet we observed the opposite pattern. One explanation is that selection against inbred individuals is particularly strong in the northern populations, as inbreeding depression is more severe in more harsh environments (Miller, 1984). Alternatively, the differences could arise via kin selection in southern populations, or due to differing levels of multiple mating, as implied by marked divergence of male reproductive traits in these same populations (Rudin-Bitterli, 2018), and recognition of complex and variable mating systems in congeners (O'Brien et al., 2018).

Signatures of local adaptation

A genome-wide scan identified 413 outlier loci putatively under directional selection. Soil moisture and rainfall variables were correlated with the greatest number of outlier loci, suggesting these factors are strong drivers of local adaptation in *P. guentheri*. Evaporation is likely to impose selection pressure as it determines the length of time standing water is available between rainfall events and affects soil moisture. Rainfall is an important trigger of breeding, initiates hatching and provides the ephemeral water sources that allow the completion of metamorphosis (Bradford & Seymour, 1988). Unsurprisingly, locations with lower rainfall tend to have drier soils, higher evaporation and higher temperatures (Table S5). In concert, these variables potentially create intense selection, likely influencing embryonic mortality, the time taken to hatch (induced when nest sites flood), the rate of larval development and the survival of metamorphs (Blaustein et al., 2010; Donnelly & Crump, 1998; Eads et al., 2012).

One locus identified as being under directional selection (correlated with both evaporation and soil moisture) resulted in a high blast match with an mRNA transcript of Protein Kinase C, zeta (PKC). This suggests local adaptation of populations in a genic region involved in the activation of oocyte maturation. Specifically, the zeta isoform of PKC is expressed in *Xenopus* oocytes in early cell division (Gosner stage 6; (Gosner, 1960) and is critically important in the activation of oocyte maturation and in the control of proliferative cascades (Dominguez et al., 1992). The mechanisms that control PKC zeta activity are poorly characterized, but activation of this protein by Ras (a family of proteins that are involved in mediating the early cellular response to mitogens) has been suggested (Diaz-Meco et al., 1994).

In *P. guentheri* there is likely to be positive selection on marginal populations for faster oocyte maturation, in response to less frequent and less predictable rainfall (Nicholls, Drosdowsky, & Lavery, 1997). Variation within the genic region of PKC zeta may be associated with differential expression of the PKC zeta gene (Liu et al., 2004), which could result in oocyte cell proliferation at variable rates. Hence, northern populations may be locally adapted to express PKC zeta at higher levels for faster

oocyte maturation to exploit unpredictable rainfall events for breeding. Further research is required to identify the function of different PKC zeta alleles in amphibian oocytes and to compare their expression levels (temporally and quantitatively) from populations adapted to different environments. Although this was the only genic region we detected in *P. guentheri* linked to local adaptation, there are likely to be more given the very strong correlations between outlier loci and environmental variables. These include genes involved in the hatching response (for which there is evidence of heritability in this species; Eads et al. 2012), and genes that influence larval development rates. Essentially, limited genomic resources for amphibian species (currently four complete genomes), and their phylogenetic divergence from *P. guentheri* (pairwise divergences of 148-205 MYA; http://www.timetree.org/), reduces the likelihood that functional genes under selection can be identified.

Two of the fourteen species currently recognized in the Australian amphibian genus *Pseudophryne* are listed as Critically Endangered, and a third as Vulnerable (EPBC Act, 1999). While *P. guentheri* is not threatened, our analysis reveals genetic patterns consistent with population isolation, decline and inbreeding, suggesting the species is at greater risk of extinction than is currently recognised. We provide strong molecular evidence of local adaption, which is supported by a parallel study of *P. guentheri* that found strong patterns of clinal variation in desiccation tolerance (both in adults and first generation offspring) across a rainfall gradient (Rudin-Bitterli, 2018). Limited gene flow between populations means that much of the variation that exists at the species level is unavailable to populations threatened by environmental change. To enhance the resilience of this species to a rapidly changing climate, targeted gene flow could potentially be used to improve its adaptive capacity. Targeted gene flow comes with its own set of risks, the major one being outbreeding depression. This has been highlighted by outcrossing different populations of the common frog *Rana temporaria*, which resulted in malformed offspring (Sagvik, Uller, & Olsson, 2005). Caution should be taken in implementing such a strategy, however, targeted gene flow may be necessary to demographically and adaptively rescue highly inbred populations (Macdonald, Llewelyn, Moritz, & Phillips, 2017). In theory, alleles suited to hotter temperatures and drier climates could be introduced to more

mesic *P. guentheri* populations, either via managed movement of individuals or their gametes from different populations (Carlson *et al*. 2014). We recommend in particular that marginal populations to the north and east of the range are monitored and protected, as they are likely to contain alleles that could benefit the broadest suite of mesic populations that may soon be maladapted to their local environments.

Acknowledgements

We thank Paul Doughty, Rebecca Bray and Ryan Ellis from the Western Australian Museum for provision and registration of tissue samples, Daniel White, Oliver Berry and Blair Bentley for advice on genetic analyses, and Yvette Hitchen for assistance in the laboratory and Adrzej Kilian, Kasia Heller and Cina Zachariah from Diversity Arrays Technology for generating the SNP data and advice on tissue sample preparation. Stewart Macdonald, Brighton Downing and Marcus Lee were instrumental in collecting many of the 2016 samples. Funding was provided by the Australian Government's National Environmental Science Programme through the Threatened Species Recovery Hub, and the School of Biological Sciences at The University of Western Australia.

We certify that we have no affiliation or involvement with any organization with financial or non-financial interest in the subject matter discussed in this manuscript.

Data Accessibility Statement

Environmental data including yearly averages (1980 - 2017) of soil moisture, rainfall, temperature and evaporation, for the coordinates of each population and SNP data will be deposited in the Dryad Digital Repository.

Author Contributions

JK and NM conceived the study, TBR and NM provided tissue samples, DC and JK analysed the data and DC drafted the manuscript. All authors wrote the final version of the manuscript.

References

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications, 1*(1), 95-111. doi:10.1111/j.1752-4571.2007.00013.x

Alonso, G. (1975). Distances between populations of Drosophila subobscura, based on chromosome arrangements frequencies. *Theoretical and Applied Genetics, 45*, 231-241.

Andersen, L. W., Fog, K., & Damgaard, C. (2004). Habitat Fragmentation Causes Bottlenecks and Inbreeding in the European Tree Frog (*Hylaarborea*). *Biological Sciences, 271*(1545), 1293-1302.

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). Lositan: a workbench to detect molecular adaptation based on a Fst -outlier method. *BMC Bioinformatics, 9*, 1–5.

Auld, J. R., Agrawal, A. A., & Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B, 277*(1681), 503-511. doi:10.1098/rspb.2009.1355

Barrett, R. D., H,, , & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology and Evolution, 23*, 38-44.

Bates, B. C., Hope, P., Ryan, B., Smith, I., & Charles, S. (2008). Key Findings from the Indian Ocean Climate Initiative and their impact on policy development in Australia. *Climate Change, 89*, 339-354.

Bauer, D. F. (1972). Constructing confidence sets using rank statistics. *Journal of the American Statistical Association, 67*, 687-690.

Beebee, T. J. (1995). Amphibian breeding and climate. *Nature, 374*, 219-220.

Beebee, T. J., & Griffiths, R. A. (2005). The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation, 125*, 271-285. doi:10.1016/j.biocon.2005.04.009

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics, 29*, 1165-1188.

Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to Climate Change in Coastal Marine Ecosystems. *Annual Review of Marine Science, 5*, 371-392. doi:10.1146/annurev-marine-121211-172411

618 Bijlsma, R., Bundgaard, J., & Van Putten, W. F. (1999). Environmental dependence of
619 inbreeding depression and purging in *Drosophila melanogaster*. *Journal of*
620 *Evolutionary Biology, 12*(6), 1125-1137. doi:10.1046/j.1420-9101.1999.00113.x
621 Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L., & Gervasi, S. S.
622 (2010). Direct and indirect effects of climate change on amphibian populations.
623 *Diversity, 2*(2), 281-313.
624 Bradford, D. F., & Seymour, R. S. (1988). Influence of Water Potential on Growth and
625 Survival of the Embryo, and Gas Conductance of the Egg, in a Terrestrial Breeding
626 Frog, *Pseudophryne bibroni*. *Physiological Zoology, 61*(5), 470-474.
627 Burel, F., Baudry, J., Butet, A., et al. (1998). Comparative biodiversity along a gradient of
628 agricultural landscapes. *Acta Oecologica, 19*(1), 47-60.
629 Burns, E. L., Eldridge, M. D., & Houlden, B. A. (2004). Microsatellite variation and
630 population structure in a declining Australian *Hylid Litoria* aurea. *Molecular Ecology,*
631 *13*(7), 1745-1757. doi:10.1111/j.1365-294X.2004.02190.x
632 Charlesworth, B. (1998). Measures of divergence between populations and the effect of
633 forces that reduce variability. *Molecular Biology and Evololution, 15*(5), 538-543.
634 Collins, J. P., & Storfer, A. (2003). Global amphibian declines: sorting the hypotheses.
635 *Diversity and Distributions, 9*, 89-98.
636 Dewitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity.
637 *Trends in Ecology & Evolution, 13*(2), 77-81. doi:10.1016/S0169-5347(97)01274-3
638 Diaz-Meco, M. T., Lozano, J., Municio, M. M., et al. (1994). Evidence for the in vitro and in
639 vivo interaction of Ras with protein kinase C zeta. *Journal of Bilogical Chemistry,*
640 *269*(50), 31706-31710.
641 Dixo, M., Metzger, J. P., Morgante, J. S., & Zamudio, K. R. (2009). Habitat fragmentation
642 reduces genetic diversity and connectivity among toad populations in the Brazilian
643 Atlantic Coastal Forest. *Biological Conservation, 142*(8), 1560-1569.
644 doi:10.1016/j.biocon.2008.11.016
645 Dominguez, I., Diaz-Meco, M. T., Municio, M. M., et al. (1992). Evidence for a role of
646 protein kinase C zeta subspecies in maturation of *Xenopus laevis* oocytes.
647 *Molecular and Cellular Biology, 12*(9), 3776. doi:10.1128/MCB.12.9.3776

Donnelly, M., & Crump, M. (1998). Potential Effects of Climate Change on Two Neotropical Amphibian Assemblages. *Climatic Change, 39*(2), 541-561. doi:10.1023/A:1005315821841

Driscoll, D. A. (1997). Mobility and metapopulation structure of *Geocrinia alba* and *Geocrinia vitellina*, two endangered frog species from southwestern Australia. *Australian Journal of Ecology, 22*, 185-195.

Driscoll, D. A. (1998). Genetic structure, metapopulation processes and evolution influence the conservation strategies for two endangered frog species. *Biological Conservation, 83*(1), 43-54.

Eads, A. R., Mitchell, N. J., & Evans, J. P. (2012). Patterns of genetic variation in desiccation tolerance in embryos of the terrestrial-breeding frog, *Pseudophryne guentheri*. *Evolution, 66*(9), 2865-2877. doi:10.1111/j.1558-5646.2012.01616.x

Earl, D., & vonHoldt, B. (2012). Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources, 4*(2), 359-361. doi:10.1007/s12686-011-9548-7

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology, 17*(5), 1170-1188. doi:10.1111/j.1365-294X.2007.03659.x

Ellstrand, N. C., & Elam, D. R. (1993). Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics, 24*, 217-242.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure : a simulation study. *Molecular Ecology, 14*(8), 2611-2620. doi:10.1111/j.1365-294X.2005.02553.x

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics, 180*(2), 977-993. doi:10.1534/genetics.108.092221

Frankham, R. (1995). Conservation Genetics. *Conservation Genetics, 29*, 305-327.

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution, 6*(8), 925-929. doi:10.1111/2041-210X.12382

679 Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and
680 efficient estimation of individual ancestry coefficients. *Genetics, 196*(4), 973-983.
681 doi:10.1534/genetics.113.160572
682 Friedman, M. (1937). The use of ranks to avoid the assumption of normality implicit in the
683 analysis of variance. *Journal of the American Statistical Association, 32*(200), 675-
684 701.
685 Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A., & Merila, J. (2008). Climate change and
686 evolution: disentangling environmental and genetic responses. *Molecular Ecology,*
687 *17*(1), 167-178. doi:10.1111/j.1365-294X.2007.03413.x
688 Gosner, K. L. (1960). A Simplified Table for Staging Anuran Embryos and Larvae with
689 Notes on Identification. *Herpetologica, 16*(3).
690 Goudet, J. (2005). Hierefstat, a package for R to compute and test hierarchical F-statistics.
691 *Molecular Ecology, 5*, 184-186.
692 Gray, M., Smith, L., & Leyva, R. (2004). Influence of agricultural landscape structure on a
693 Southern High Plains, USA, amphibian assemblage. *Landscape Ecology, 19*(7),
694 719-729. doi:10.1007/s10980-005-1129-3
695 Heap, S. M., Stuart-Fox, D., & Byrne, P. G. (2015). Reduction in site fidelity with smaller
696 spatial scale may suggest scale-dependent information use. *Behavioural Ecology,*
697 *26*(2), 543-549.
698 Hobbs, R. J. (1993). Effects of landscape fragmentation on ecosystem processes in the
699 Western Australian wheatbelt. *Biological Conservation, 64*(3), 193-201.
700 doi:10.1016/0006-3207(93)90321-Q
701 Holzhauer, S. I. J., Ekschmitt, K., Sander, A., Dauber, J., & Wolters, V. (2006). Effect of
702 historic landscape change on the genetic structure of the bush-cricket *Metrioptera*
703 *roeseli*. *Landscape Ecology, 21*(6), 891-899.
704 Jarvis, N. (1986). *Western Australia: An Atlas of Human Endeavour*. Perth, Western
705 Australia: Department of Lands and Surveys.
706 Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal
707 components: a new method for the analysis of genetically structured populations.
708 *BMC Genet, 11*, 94. doi:10.1186/1471-2156-11-94

709 Kamvar, Z. N., Tabima , J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic
710 analysis of populations with clonal, partially clonal, and/or sexual reproduction.
711 *PeerJ*. doi:10.7717/peerj.281
712 Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in*
713 *Ecology & Evolution, 17*(5), 230-241. doi:10.1016/S0169-5347(02)02489-8
714 Kilian, A., Wenzl, P., Huttner, E., et al. (2012). *Diversity Arrays Technology: A Generic*
715 *Genome Profiling Technology on Open Platforms* (Vol. 888): Humana Press,
716 Totowa, NJ.
717 Krzanowski, W. J. (1987). Cross-Validation in Principal Component Analysis. *Biometrics,*
718 *43*(3), 575-584.
719 Landguth, E. L., Cushman, S. A., Schwartz, M. K., McKelvey, K. S., Murphy, M., & Luikart,
720 G. (2010). Quantifying the lag time to detect barriers in landscape genetics.
721 *Molecular Ecology, 19*(19), 4179-4191.
722 Lawler, J. J., Shafer, S. L., Bancroft, B. A., & Blaustein, A. R. (2010). Projected climate
723 impacts for the amphibians of the Western hemisphere. *Conservation Biology,*
724 *24*(1), 38-50. doi:10.1111/j.1523-1739.2009.01403.x
725 Lenhardt, P. P., Brühl, C. A., Leeb, C., & Theissinger, K. (2017). Amphibian population
726 genetics in agricultural landscapes: does viniculture drive the population structuring
727 of the European common frog (Rana temporaria)? *PeerJ, 5*, e3520-e3520.
728 doi:10.7717/peerj.3520
729 Levy, E., Kennington, W. J., Tomkins, J. L., & Lebas, N. R. (2010). Land clearing reduces
730 gene flow in the graniteoutcrop-dwelling lizard, *Ctenophorus ornatus*. *Molecular*
731 *Ecology, 19*, 4192–4203
732 Levy, E., Tomkins, J. L., Lebas, N. R., & Kennington, W. J. (2013). Contrasting effects of
733 landscape features on genetic structure in different geographic regions in the ornate
734 dragon lizard, *Ctenophorus ornatus*. *Molecular Ecology, 22*(15), 3904-3915.
735 doi:10.1111/mec.12367
736 Lips, K. R., Diffendorfer, J., Mendelson, J. R., & Sears, M. W. (2008). Riding the wave:
737 reconciling the roles of disease and climate change in amphibian declines. *PLOS*
738 *Biology, 6*(3), 441-454. doi:10.1371/journal.pbio.0060072

739 Liu, Z., Sun, H., Zhang, Y., et al. (2004). Effect of SNPs in protein kinase Cz gene on gene
740 expression in the reporter gene detection system. *World Journal of*
741 *Gastroenterology, 10*(16), 2357-2360.
742 Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: an R package to perform genome
743 scans for selection based on principal component analysis. *Molecular Ecology*
744 *Resources, 17*(1), 67-77. doi:10.1111/1755-0998.12592
745 Macdonald, S. L., Llewelyn, J., Moritz, C., & Phillips, B. L. (2017). Peripheral Isolates as
746 Sources of Adaptive Diversity under Climate Change. *Frontiers in Ecology and*
747 *Evolution, 5*(88).
748 Melville, J., Haines, M. L., Boysen, K., et al. (2017). Identifying hybridization and admixture
749 using SNPs: application of the DArTseq platform in phylogeographic research on
750 vertebrates. *Royal Society Open Science, 4*(7), 161061. doi:10.1098/rsos.161061
751 Miller, P. (1984). Is inbreeding depression more severe in a stressful environment? . *Zoo*
752 *Biology, 13*(3).
753 Mitchell, N. J. (2001). Males call more from wetter nests: effects of substrate water
754 potential on reproductive behaviours of terrestrial toadlets. *Proceedings of the*
755 *Royal Society B: Biological Sciences, 268*(1462), 87-93.
756 doi:10.1098/rspb.2000.1334
757 Mitchell, N. J. (2005). Nest swapping in an Australian toadlet (*Pseudophryne bibroni*): do
758 males respond to chemical signals? *Herpetological Review, 36*(1), 19-21.
759 Narum, S. R., & Hess, J. E. (2011). Comparison of FST outlier tests for SNP loci under
760 selection. *Molecular Ecology Resources, 11*(1), 184-194. doi:10.1111/j.1755-
761 0998.2011.02987.x
762 Nei, M. (1972). Genetic distances between populations. *American Naturalist, 106*, 283-
763 292.
764 Nicholls, N., Drosdowsky, W., & Lavery, B. (1997). Australian rainfall variability and
765 change. *Weather, 52*, 66-72.
766 O'Brien, D. M., Keogh, J. S., Silla, A. J., & Byrne, P. G. (2018). The unexpected genetic
767 mating system of the red-backed toadlet (Pseudophryne coriacea): A species with
768 prolonged terrestrial breeding and cryptic reproductive behaviour. *Molecular*
769 *Ecology, 27*(14), 3001-3015. doi:doi:10.1111/mec.14737

Pearson, W. R. (2013). An introduction to sequence similarity ("homology") searching. *Current protocols in bioinformatics, Chapter 3*, Unit3.1-Unit3.1. doi:10.1002/0471250953.bi0301s42

Perl, R. G. B., Gafny, S., Malka, Y., et al. (2017). Natural history and conservation of the rediscovered Hula painted frog, Latonia nigriventer. *Contributions to Zoology, 86*(1), 11-37.

Pounds, A. J., Fogden, M. P. L., & Campbell, J. H. (1999). Biological response to climate change on a tropical mountain. *Nature, 398*, 611-615.

Prevosti, A. (1974). La distancia genetica entre poblaciones. *Miscellanea Alcobe, 68*, 109-118.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Reed, D., Lowe, E., Briscoe, D., & Frankham, R. (2003). Inbreeding and extinction: Effects of rate of inbreeding. *Conservation Genetics, 4*(3), 405-410. doi:10.1023/A:1024081416729

Richter, S. C., Crother, B. I., Broughton, R. E. (2009). Genetic Consequences of Population Reduction and Geographic Isolation in the Critically

Endangered Frog, *Rana sevosa*. *Copeia, 2009*(4), 799-806.

Robertson, A. (1965). The interpretation of genotypic ratios in domestic animal populations. *Animal Science, 7*(3), 319-324. doi:10.1017/S0003356100025770

Roff, A. (1998). Effects of inbreeding on morphological and life history traits of the sand cricket, *Gryllus firmus*. *Heredity, 81*(1), 28.

Rudin-Bitterli, T. S., Evans, J. P., & Mitchell, N. J. (2018). Geographic variation in adult and embryonic desiccation tolerance in a terrestrial-breeding frog. *bioRxiv, 314351*.

Sagvik, J., Uller, T., & Olsson, M. (2005). Outbreeding depression in the common frog, *Rana temporaria*. *Conservation Genetics, 6*, 205-211. doi:10.1007/s10592-004-7829-3

Sánchez-Sevilla, J. F., Horvath, A., Botella, M. A., et al. (2015). Diversity Arrays Technology (DArT) Marker Platforms for Diversity Analysis and Linkage Mapping in a Complex Crop, the Octoploid Cultivated Strawberry (*Fragaria* × *ananassa*). *PLoS One, 10*(12), e0144960. doi:10.1371/journal.pone.0144960

Saunders, D. A. (1989). Changes in the Avifauna of a region, district and remnant as a result of fragmentation of native vegetation: the wheatbelt of western Australia. A case study. *Biological Conservation, 50*(1), 99-135. doi:10.1016/0006-3207(89)90007-4

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications, 4*(2), 326-337. doi:10.1111/j.1752-4571.2010.00157.x

Slatkin, M. (1987). Gene Flow and the Geographic Structure of Natural Populations. *Science, 236*(4803), 787-792. doi:10.1126/science.3576198

Slatkin, M., & Excoffier, L. (2012). Serial founder effects during range expansion: a spatial analog of genetic drift. *Genetics, 191*(1), 171-181. doi:10.1534/genetics.112.139022

Smith, A. M., & Green, D. M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography, 28*, 110-128.

Spear, S. F., & Storfer, A. (2008). Landscape genetic structure of coastal tailed frogs (Ascaphus truei) in protected vs. managed forests. *Molecular Ecology, 17*, 4642-4656.

Spielman, D., Brook, B., & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States, 101*(42), 15262-15264.

Thomas, L., Kennington, W. J., Evans, R. D., Kendrick, G. A., & Stat, M. (2017). Restricted gene flow and local adaptation highlight the vulnerability of high-latitude reefs to rapid environmental change. *Global Change Biology, 23*(6), 2197-2205. doi:10.1111/gcb.13639

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology, 25*(10), 2144-2164. doi:10.1111/mec.13606

Todd, B. D., & Winne, C. T. (2006). Ontogenetic and interspecific variation in timing of movement and responses to climatic factors during migrations by pond-breeding amphibians. *Canadian Journal of Zoology, 84*(5), 715-722. doi:10.1139/z06-054

Urban, M. C., Richardson, J. L., & Freidenfelds, N. A. (2014). Plasticity and genetic adaptation mediate amphibian and reptile responses to climate change. *Evolutionary Applications, 7*(1), 88-103. doi:10.1111/eva.12114

Van Rooij, P., Martel, A., Haesebrouck, F., & Pasmans, F. (2015). Amphibian chytridiomycosis: a review with focus on fungus-host interactions. *Veterinary Research, 46*, 137. doi:10.1186/s13567-015-0266-0

Villemereuil, P., & Gaggiotti, O. E. (2015). A new FST‐based method to uncover local adaptation using environmental variables. *Methods in Ecology and Evolution, 6*(11), 1248-1258. doi:10.1111/2041-210X.12418

Wake, D. B. (2012). Facing extinction in real time. *Science, 335*, 1052-1053. doi:10.1126/science.1218364

Wake, D. B., & Vredenburg, V. T. (2008). Colloquium paper: are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America, 105* (1), 11466. doi:10.1073/pnas.0801921105

Walls, S. C., Barichivich, W. J., & Brown, M. E. (2013). Drought, deluge and declines: the impact of precipitation extremes on amphibians in a changing climate. *Biology, 2*(1), 399-418. doi:10.3390/biology2010399

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution, 38*(6), 1358-1370.

Weller, R. (2009). *Boomtown 2050 : scenarios for a rapidly growing city*. Crawley, WA.

Winter, M., Fiedler, W., Hochachka, W. M., Koehncke, A., Meiri, S., & De la Riva, I. (2016). Patterns and biases in climate change research on amphibians and reptiles: a systematic review. *Royal Society Open Science, 3*(9), 160-158.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics, 16*, 97–158.

Tables

Table 1 Sequence matches with genic regions recovered for outlier loci.

Locus	Reference species	Matching region	Percentage coverage (%)	E - value
9309	*Salmo salar*	Upstream of Chromatin modifying protein (Chmp2a), EU025709.1	100	3.00E-18
10128	*Xenopus tropicalis*	Predicted: protein kinase C, zeta mRNA (variants X1, X2, X3), XM_012965862	82	1.00E-11
10128	*Xenopus laevis*	Predicted: protein kinase C, zeta mRNA (variants X1, X3, X4, X5, X6, X7, X8), XM_018227867.1	82	6.00E-10

Figures

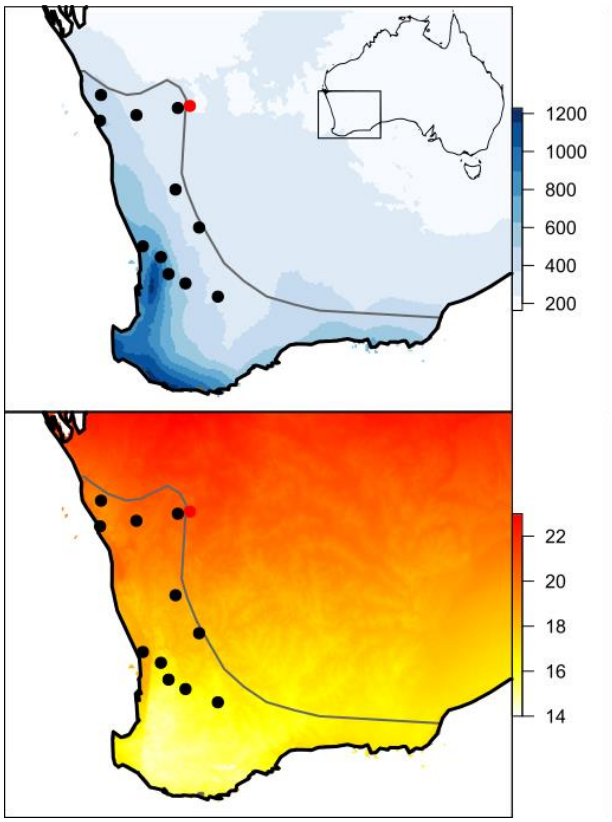

Fig. 1 *Pseudophryne guentheri* sample locations overlaid on average annual precipitation (mm) and temperature (°C) data respectively (top to bottom). The red point shows the location of the *Pseudophryne occidentalis* population used in this study, while the grey line shows the approximate eastern edge of the range of *P. guentheri.*

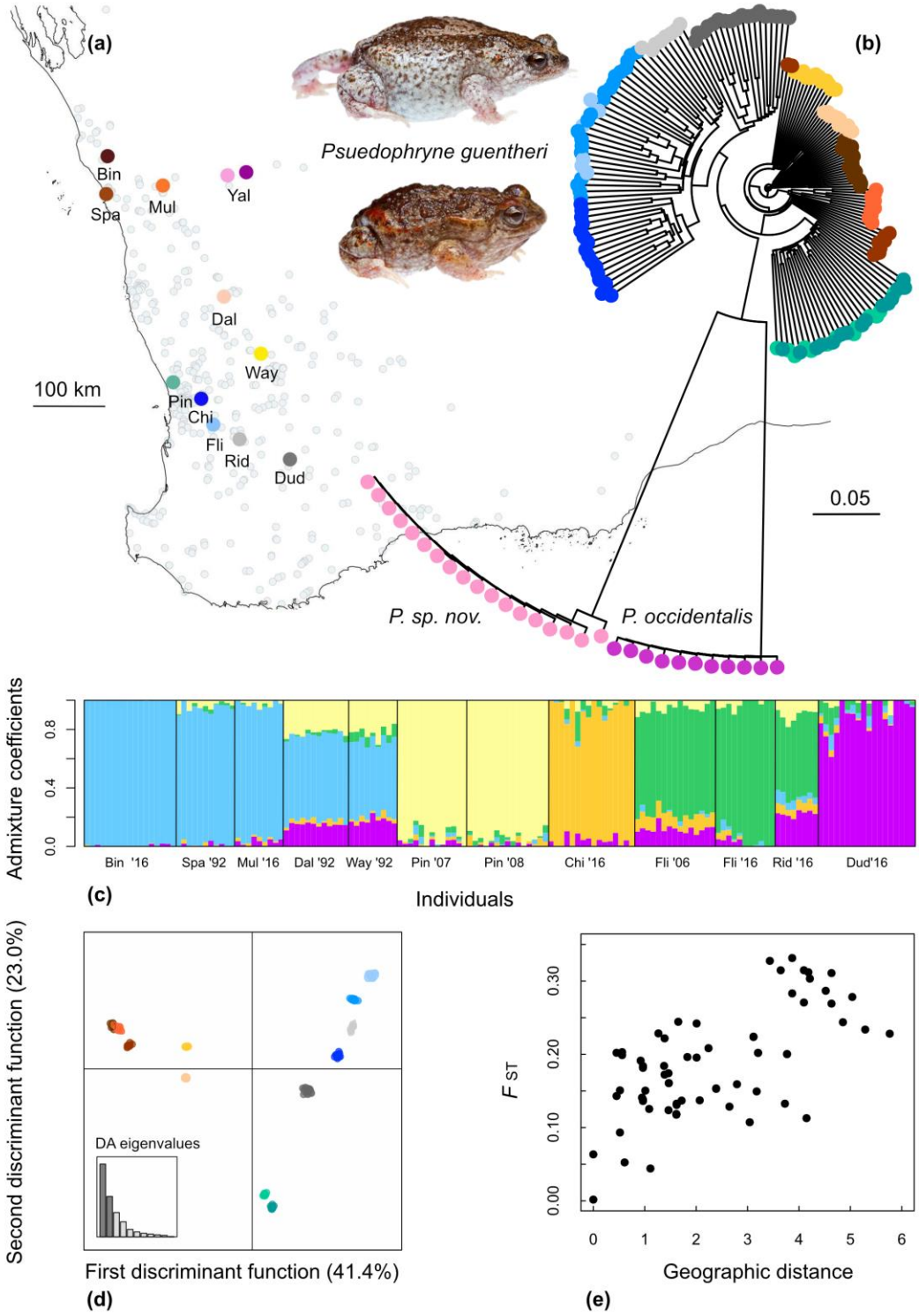

Fig. 2 Descriptive statistics showing population samples and different aspects of *P. guentheri* genetic structure. a) Locations of sampled *P. guentheri* and *P. occidentalis* populations (coloured circles) overlaid atop all distribution records *for P. guentheri* (grey circles; data from http://spatial.ala.org.au). Population abbreviations are outlined in Table S5. b)

Neighbour joining tree based on Nei's genetic distance using all individuals (including *P. occidentalis* and a cryptic species). c) Bar chart of ancestry coefficients from a sNMF analysis of *P. guentheri* samples (K = 5) based on neutral loci, and d), a DAPC scatter plot of the same data. e) The relationship between pairwise F_{ST} (neutral loci) and geographic distance (as a distance matrix) for population samples (r = 0.649, P < 0.001). *P. guentheri* photo credit: Corne van Linden.

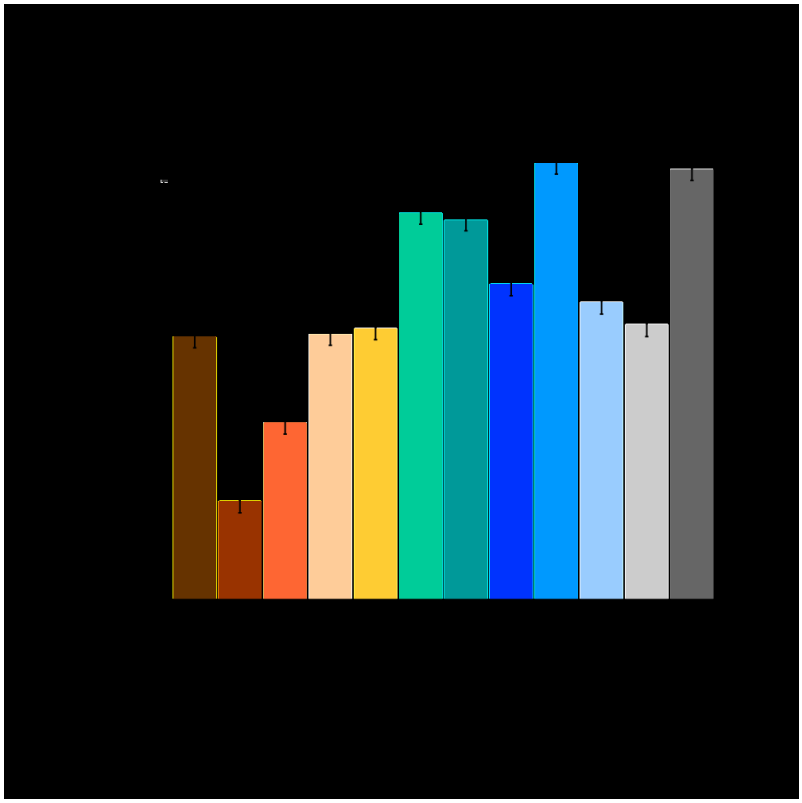

Fig. 3 Mean expected heterozygosity calculated using selectively neutral loci (±SE). Letters denote statistically different groups (Table S3) based on pairwise significant differences, calculated using BY corrected p-values (P < 0.00076). Colours correspond to those used in Fig. 2.

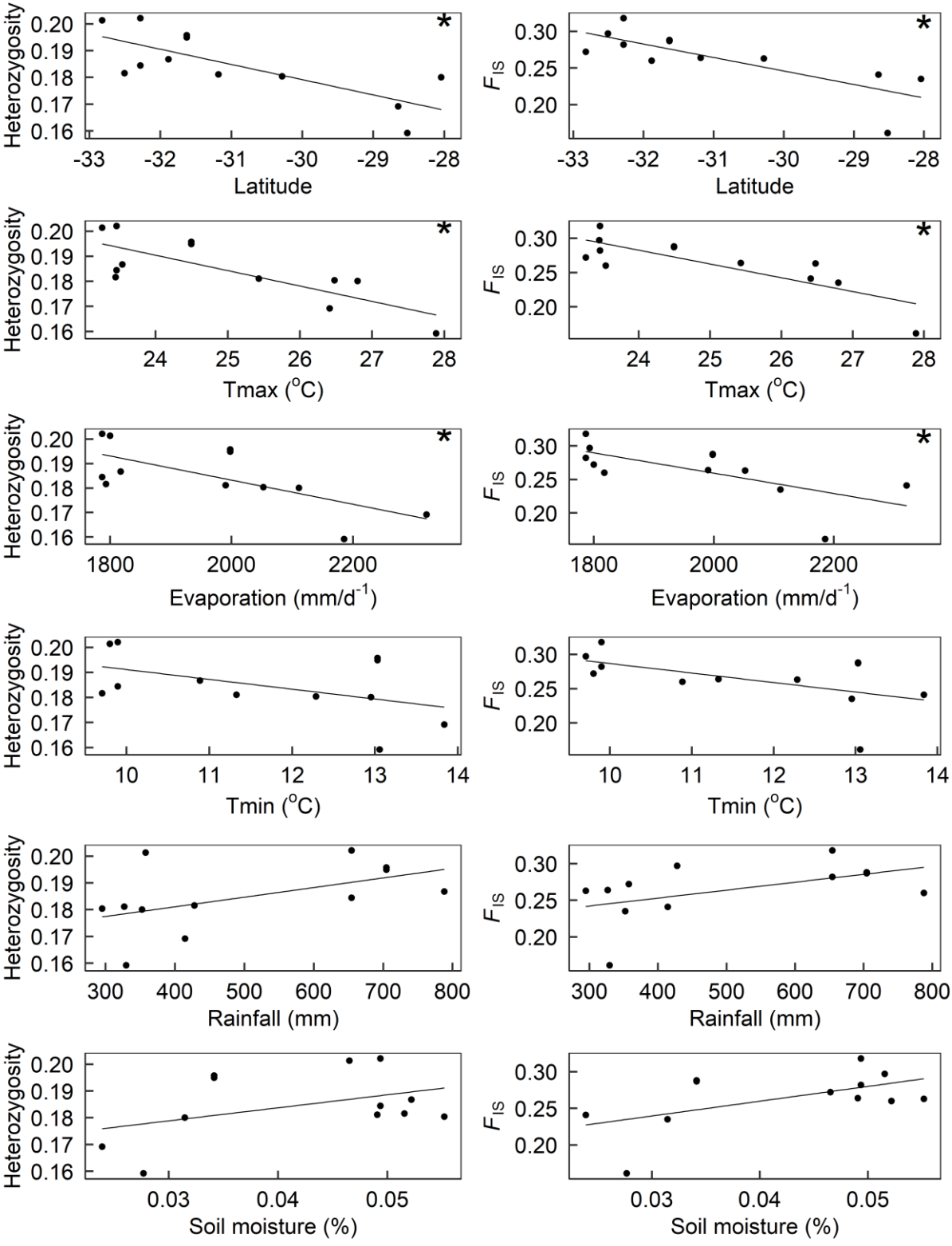

Fig. 4 Linear relationships between estimates of genetic variation (left column) or inbreeding coefficients (F_{IS}) (right column) and latitude plus five environmental variables. Significant relationships are denoted by an asterisk (Table S2).

907