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Fish out of water: Genomic insights into persistence of rainbowfish populations in the desert

Catherine R. M. Attard,[1] Jonathan Sandoval-Castillo,[1] Chris J. Brauer,[1] Peter J. Unmack,[2] David Schmarr,[3] Louis Bernatchez,[4] and Luciano B. Beheregaray[1,5]

[1]*Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Adelaide, SA 5001, Australia*

[2]*Centre for Applied Water Science, Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia*

[3]*Inland Waters and Catchment Ecology Program, SARDI Aquatic Sciences, Henley Beach, SA 5022, Australia*

[4]*Institut de Biologie Intégrative et des Systèmes, Université Laval Québec, Québec, QC G1V 0A6, Canada*

[5]*E-mail: Luciano.Beheregaray@flinders.edu.au*

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How populations of aquatic fauna persist in extreme desert environments is an enigma. Individuals often breed and disperse during favorable conditions. Theory predicts that adaptive capacity should be low in small populations, such as in desert fishes. We integrated satellite-derived surface water data and population genomic diversity from 20,294 single-nucleotide polymorphisms across 344 individuals to understand metapopulation persistence of the desert rainbowfish (*Melanotaenia splendida tatei*) in central Australia. Desert rainbowfish showed very small effective population sizes, especially at peripheral populations, and low connectivity between river catchments. Yet, there was no evidence of population-level inbreeding and a signal of possible adaptive divergence associated with aridity was detected. Candidate genes for local adaptation included functions related to environmental cues and stressful conditions. Eco-evolutionary modeling showed that positive selection in refugial subpopulations combined with connectivity during flood periods can enable retention of adaptive diversity. Our study suggests that adaptive variation can be maintained in small populations and integrate with neutral metapopulation processes to allow persistence in the desert.

KEY WORDS: **Adaptive resilience, arid zone, climate change, freshwater fish, landscape genomics, metapopulation.**

Life manages to persist in unpredictable and extreme environmental niches. How it persists is a major evolutionary question that has fascinated people for decades (Rothschild and Mancinelli 2001). In the case of obligate aquatic organisms, extreme environments include those with little water such as the deserts of central Australia, parts of North and South America, northern and southern Africa, and the Middle East to central Asia (Peel et al. 2007). These dry regions are predicted to expand in future climate scenarios (Huang et al. 2015) and to have more extreme climatic fluctuations (Leigh et al. 2015). This makes understanding the persistence of aquatic life in the desert a key question for how organisms may survive (or not) under future climate change.

Answering this question has focused on neutral population demographics (see review Murphy et al. 2015). Many desert species opportunistically breed and disperse when there are favorable conditions and hydrological connectivity, such as during rare flooding events. These high dispersal species act as cyclical boom-bust metapopulations, with local extinction during dry periods and recolonization in wet periods (Huey et al. 2011; Agnèse et al. 2018). Their level of population connectivity is limited by riverine connectivity, with low or nil population structure within river catchments, moderate structure among catchments in the same basin (i.e., catchments that flow to a common outlet), and relatively high structure among river basins (Murphy et al. 2015). At the opposite end of the spectrum, low dispersal species

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occupy permanent habitats rather than exploring unknown river areas (e.g., Carini and Hughes 2006; Bernardi et al. 2007). Such species can show divergence of lineages even among refuges that are separated by only a few hundred meters. Desert hydrology additionally varies widely, from permanent springs and waterholes, to seasonal rainfall regimes and intermittent rivers and streams with highly unpredictable boom-and-bust cycles and associated floodplains (e.g. Morton et al. 2011; Schriever et al. 2015). Aquatic life in extreme aridity is therefore strongly linked with life history strategies and the hydrological attributes of the arid region.

It is uncertain how these ecological processes interact with adaptive evolution. Desert-dwelling species have morphological, physiological, and behavioral adaptations to aridity—such as their dispersal behaviors—but evolutionary processes operate on a population level and desert hydrology is highly heterogeneous. Adaptive evolution is conceivably hindered by the population demographics of aquatic obligates in the desert. Species that occupy small permanent water bodies will have the benefit of stable habitat, but at the cost of a low population size, increased genetic drift, and associated reduced strength of natural selection and therefore adaptive evolution. There is an increasing number of studies showing that small population size may not inherently mean poor adaptive capacity (Wood et al. 2016; Fraser 2017; Perrier et al. 2017), but we do not know when and how this occurs. At the opposite end of the spectrum, the high connectivity of dispersers reduces the influence of natural selection through the homogenizing effect of gene flow. Adaptive evolution despite gene flow has already been found in nondesert regions (Sanford and Kelly 2011; Jones et al. 2012; Pavey et al. 2015). This may enable population persistence in the desert as gene flow inflates effective population size, thereby maintains standing genetic variation, and allows potentially adaptive alleles in one region to spread and be selected for in newly altered habitats (Attard et al. 2018).

Rainbowfishes (genus *Melanotaenia*) are an emerging Australian system to study hydroclimatically driven adaptive evolution (McGuigan et al. 2003; Smith et al. 2013; Unmack et al. 2013; McCairns et al. 2016; Gates et al. 2017; Brauer et al. 2018; Sandoval-Castillo et al. 2020; Smith et al. 2020). Here, we integrate high-resolution, satellite-derived surface water data with genome-wide variation and eco-evolutionary simulations to examine the dynamics between connectivity, effective population size, and adaptive diversity in an aquatic obligate of the desert, the desert rainbowfish (*Melanotaenia splendida tatei*). This species is a small (maximum 10 cm length) fish that spans across the extremely unstable (i.e., hydroclimatically variable) peripheral rivers in the arid west of central Australia and the comparatively stable semi-arid habitat in the east (Fig. 1). Desert rainbowfish populations experience cyclical boom-bust periods driven by the largely dry but unpredictable rainfall regime of Australia's

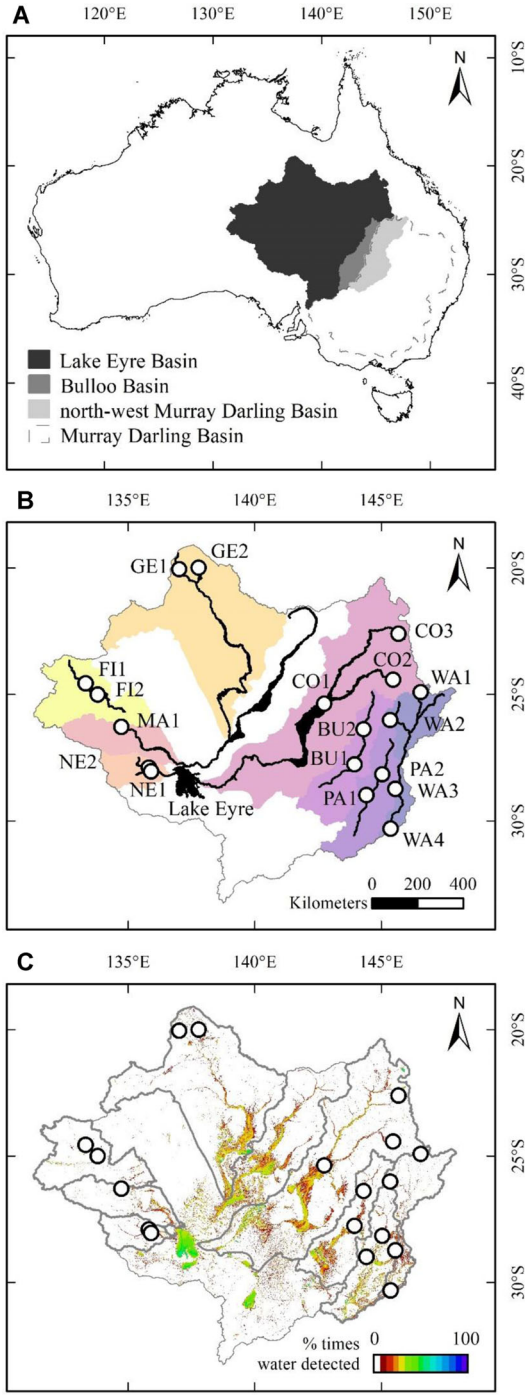

Figure 1. Maps of desert rainbowfish distribution range, sampling, and environmental data. The maps specifically show (A) the location of Lake Eyre, Bulloo, and Murray-Daring basins in Australia, with shading of regions occupied by the desert rainbowfish; (B) zoom-in of desert rainbowfish range, with shading of the eight catchments where desert rainbowfish were sampled (colors following Fig. 2) and circles representing sampling sites (coded following Table 1); (C) zoom-in of desert rainbowfish range showing the percent of times that surface water has been observed in each 25-m resolution pixel from January 1987 to February 2016 based on *Water Observations from Space* (Mueller et al. 2016) (sampled catchments and sampling sites are also indicated).

arid zone (DeVogel et al. 2004). We predict greater connectivity among localities within the same catchment than among adjacent river basins. We also predict that catchments with greater aridity (i.e., less surface water and aquatic habitat) have smaller populations with lower neutral genetic diversity. This is in line with neutral demographic studies on aquatic obligates and hydrological connectivity in deserts (Murphy et al. 2015). We expect adaptive genetic diversity and divergence across populations to be modulated by connectivity and correlate with the degree of aridity. We also anticipate signals of natural selection operating on genes that might allow survival and reproduction during dry periods. This standing genetic variation could then be maintained with the aid of flood periods that promote connectivity and bursts in population size. Altogether, our study provides genomic insights into how eco-evolutionary dynamics could allow population persistence of an aquatic obligate in the desert.

Materials and Methods

ENVIRONMENTAL DATA COLLECTION

The desert rainbowfish ranges across three basins in the Australian arid zone: the Lake Eyre Basin, Bulloo Basin, and the north-west of the Murray-Darling Basin (Morton et al. 2011) (Fig. 1). The western catchments of Lake Eyre receive minimal rainfall input, relying on rare high-rainfall events to connect surface water refugia (Unmack 2001; Costelloe and Russell 2014). The situation is different to the east, where summer tropical rainfall from the north-east flows into the Cooper Creek, Diamantina River, and Georgina River catchments, as well as into the Bulloo and northern Murray-Darling basins (Kingsford et al. 2001; McMahon et al. 2008). However, the Cooper, Diamantina, and Georgina only reach saline Lake Eyre during rare, large flood events (Williams and Kokkinn 1988; Timms 2007). Bulloo Basin consists of the internally draining Bulloo catchment, and the Murray-Darling Basin includes the semi-arid Paroo and Warrego River catchments (Kingsford et al. 2001).

The degree of aridity across this range was quantified using a high-resolution, satellite-derived surface water dataset from the Australian Government: *Water Observations from Space (WOfS)* (http://www.ga.gov.au/scientific-topics/hazards/flood/wofs). These data are the percent of times in each 25-m resolution pixel that surface water was observed across more than 184,500 satellite images in Australia from January 1987 to February 2016 (Mueller et al. 2016). Surface water was chosen to infer aridity instead of a precipitation-based index because surface water can be generated from rainfall thousands of kilometers away and is of greater ecological relevance to aquatic obligates. *WOfS* was translated into two aridity indices for each sampled river catchment: (1) the amount of area with permanent water, defined as water observed at least 80% of the time (Mueller et al. 2016), and (2) the amount of area equivalent to water observed 100% of the time when water observations are amalgamated over the entire catchment. The former considers only *permanent* and presumably deeper surface water, and the latter *both permanent and transient* surface water.

GENOMIC DATA COLLECTION

Fin-clips or muscle tissue were collected from 351 desert rainbowfish in 18 sites across the geographic and environmental range of the taxon (Fig. 1). Two of these sites have opportunistic temporal replicates (Table 1) to ascertain whether population diversity or structure may differ across time. Samples were preserved in 70–100% ethanol, and DNA was extracted using a salting-out protocol (Sunnucks and Hales 1996). Libraries were prepared following the ddRAD protocol of Peterson et al. (2012) as modified in Sandoval-Castillo et al. (2018). In brief, the restriction enzymes SbfI-HF and MseI were used to digest genomic DNA, adapters including a 6 bp barcode were ligated, and 300–800 bp fragments were size selected. Each of 48 samples was paired-end 100 bp sequenced (HiSeq 2000) on one of eight separate lanes at the McGill University and Génome Québec Innovation Centre. Raw sequences were bioinformatically processed using a reference rainbowfish genome (Supporting Information). This produced a final, high-quality, and putatively unlinked single-nucleotide polymorphism (SNP) dataset that had a minor allele frequency of at least 0.03 and no more than 20% missing data per locus.

NEUTRAL POPULATION DYNAMICS

Genetic variation, population structure, connectivity (i.e., gene flow), and effective population size were estimated with the full, final SNP dataset. This is expected to reflect neutral processes as the full SNP dataset is dispersed across the genome and so would include relatively few adaptive loci.

Genetic variation within sampling localities and catchments was measured as percentage of polymorphic loci, observed heterozygosity (H_O), and unbiased expected heterozygosity (H_E) using ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010). The inbreeding coefficient (F_{IS}; significance assessed by 10,000 permutations) for each sampling site and catchment was also calculated using ARLEQUIN. Effective population size (N_e) was estimated for each locality and catchment using NeESTIMATOR 2.1 (Do et al. 2014), a method robust to different population demographic, marker, and sampling scenarios (Wang 2016). Singleton alleles were screened out and confidence intervals were calculated by jackknifing over loci.

Population structure and differentiation were assessed by Bayesian clustering using FastSTRUCTURE 1.0 (Raj et al. 2014) and ADMIXTURE 1.3.0 (Alexander et al. 2009)

Table 1. Genetic variation of desert rainbowfish and additional information for each sampling site at 20,254 SNPs. *n*, sample size (after filtering individuals based on missing data); AR, allelic richness; H_O, mean observed heterozygosity; H_E, mean unbiased expected heterozygosity; F_{IS}, inbreeding coefficient (all had $P > 0.05$); N_e, effective population size. In the site code, "a" and "b" represent temporal samples from the same site. In parentheses are the standard deviations for heterozosity, and 95% confidence intervals for N_e.

Basin	Catchment	Site code	Date collected	*n*	% polymorphic loci	H_O	H_E	F_{IS}	N_e
Lake Eyre	Finke			35	37	0.066 (0.142)	0.069 (0.142)	–0.024	55 (28–248)
		FI1	November 2011	15	31	0.073 (0.156)	0.073 (0.146)	–0.042	98 (42–∞)
		FI2	NA 1988	20	26	0.059 (0.139)	0.064 (0.142)	0.000	231 (76–∞)
Lake Eyre	Georgina			40	58	0.152 (0.187)	0.164 (0.193)	0.022	481 (233–∞)
		GE1	July 1997	20	55	0.159 (0.198)	0.167 (0.194)	0.006	327 (140–∞)
		GE2	NA 1987	20	49	0.145 (0.191)	0.159 (0.196)	0.034	558 (83–∞)
Lake Eyre	Neales			45	76	0.210 (0.182)	0.229 (0.187)	0.040	519 (299–1803)
		NE1a	November 2011	11	68	0.214 (0.210)	0.229 (0.197)	0.037	764 (209–∞)
		NE1b	September 2014	15	71	0.215 (0.201)	0.229 (0.194)	0.028	184 (74–∞)
		NE2	November 2011	19	69	0.203 (0.192)	0.225 (0.194)	0.046	445 (96–∞)
Lake Eyre	Macumba			24	77	0.236 (0.200)	0.246 (0.192)	–0.004	433 (117–∞)
		MA1a	December 2011	11	69	0.231 (0.218)	0.242 (0.201)	0.012	389 (54–∞)
		MA1b	February 2014	13	74	0.241 (0.215)	0.249 (0.197)	–0.021	420 (36–∞)
Lake Eyre	Cooper			60	79	0.230 (0.208)	0.219 (0.187)	–0.084	146 (114–199)
		CO1	May 2014	20	69	0.231 (0.223)	0.218 (0.195)	–0.090	365 (179–∞)
		CO2	June 1997	20	68	0.224 (0.225)	0.206 (0.191)	–0.118	185 (88–∞)
		CO3	May 2014	20	71	0.234 (0.227)	0.219 (0.195)	–0.123	475 (157–∞)
Bulloo	Bulloo			38	47	0.097 (0.159)	0.103 (0.163)	0.022	423 (279–855)
		BU1	May 2014	20	40	0.097 (0.164)	0.103 (0.166)	0.025	391 (151–∞)
		BU2	May 2014	18	39	0.097 (0.166)	0.102 (0.166)	0.015	444 (162–∞)
Murray-Darling	Paroo			38	54	0.092 (0.159)	0.088 (0.144)	–0.095	25 (13–59)
		PA1	May 1997	19	42	0.099 (0.178)	0.089 (0.151)	–0.139	43 (27–93)
		PA2	May 2014	19	42	0.086 (0.152)	0.085 (0.144)	–0.057	30 (17–81)
Murray-Darling	Warrego			70	70	0.123 (0.163)	0.128 (0.164)	–0.034	18 (11–31)
		WA1	June 1997	19	55	0.132 (0.176)	0.138 (0.172)	–0.044	17 (8–55)
		WA2	May 2014	16	55	0.139 (0.188)	0.136 (0.170)	–0.118	17 (7–98)
		WA3	June 1997	15	50	0.123 (0.177)	0.124 (0.169)	–0.038	25 (11–455)
		WA4	May 2014	20	34	0.102 (0.179)	0.102 (0.170)	–0.061	47 (30–96)

(default parameters), pairwise genetic differentiation (F_{ST}) and analysis of molecular variance (AMOVA) using ARLEQUIN (significance assessed by 10,000 permutations), and isolation by riverine distance using a Mantel test in GENEPOP 4.3 (significance assessed by 1000 permutations) (Raymond and Rousset 1995; Rousset 2008). The latter was only done for the Lake Eyre Basin because riverine connections and distances (calculated using ArcGIS 10.4.1) between basins are uncertain, and both the taxon and sample sites are predominantly located in the Lake Eyre Basin. Gene flow among identified populations was estimated using BAYESASS3-SNPs (Wilson and Rannala 2003) (burn-in 4×10^7 iterations then 7×10^7 iterations with sampling every 1000 iterations; mixing parameters $\Delta A = 0.3$, $\Delta F = 0.015$, $\Delta M = 0.1$; three independent runs), with runs combined using TRACER 1.7.1 (Rambaut et al. 2018). To complement the SNP dataset, we constructed a haplotype network using mitochondrial DNA (mtDNA) cytochrome *b* to assess if our samples comprise a single phylogeographic lineage (Supporting Information).

ADAPTIVE POPULATION DIVERSITY

We searched the literature for genomic studies of aquatic obligates in arid environments to determine the degree that studies of population dynamics have expanded to include adaptive diversity. This was done using all databases in Web of Science on 2 April 2021 following Murphy et al. (2015), which reviewed studies on genetic structure of aquatic fauna in arid environments. The differences between the two literature searches are we replaced "TS [i.e., topic area] = (genetic* OR connectivity OR population structure)" with "TS = (genomic* OR SNP*)" to capture genomic studies, we removed "fragment*" from the aridity search terms because it captured unrelated studies, and we allowed studies from any journal or article type.

Two complimentary genotype-environment association approaches were used in desert rainbowfish to identify genomic regions putatively under positive selection for aridity: BAYENV2 (Günther and Coop 2013) and BAYSCENV 1.1 (de Villemereuil and Gaggiotti 2015) (Supporting Information). These were chosen as they both attempt to identify population allele frequencies associated to environmental variables while controlling for population structure, but in different ways. BAYENV2 tests for covariance between genetic and environmental variation. It controls for population structure through a covariance matrix estimated between populations using the allele frequencies of all loci. BAYSCENV extends the well-known F_{ST} outlier method BAYESCAN (Foll and Gaggiotti 2008) to include possible associations with environmental and locus-specific differentiation. SNPs identified as candidates for selection were examined for potential functions through annotating 500 bp around each SNP (Supporting Information).

We also used forward genetic simulations to explore how boom-bust metapopulation dynamics and aridity-driven selection may contribute to maintaining genetic diversity in desert fish populations subject to strong drift (Supporting Information). These were based on a range of demographic parameters consistent with those inferred here for desert rainbowfish in the Lake Eyre Basin and the variable hydroclimatic regime of central Australia (DeVogel et al. 2004). We used SLiM 3.1 (Haller and Messer 2019) to model three metapopulation scenarios with subpopulation sizes of 100, 500, and 1000 individuals (Fig. S1). Following a burn-in (Fig. S2), a single subpopulation was used as the ancestral population founding three new subpopulations, for which simulations were run for an additional 20,000 generations. Population size and connectivity were varied for each of the three scenarios (subpopulation N_e = 100, 500, and 1000), including models with and without positive selection (Table S1). All simulations followed a common framework of boom-bust cycles where the small, isolated demes periodically increased in size and exchanged migrants during flood events (Fig. S1). During dry periods, there was no migration among subpopulations. Spatially heterogeneous local adaptation to aridity within drought refuges assumed that 2.5% of loci were under positive selection in subpopulation 1 during dry periods only. Scenarios were modeled for both low and high strengths of selection with each adaptive locus providing a fitness advantage of 0.001 and 0.01, respectively. For comparison, neutral simulations with no selection were also run for each scenario. One-hundred replicate runs with a different starting seed were completed for each simulation, with results used to track mean F_{ST} and H_E estimates over 20,000 generations.

Results

NEUTRAL POPULATION DYNAMICS

The genomic dataset consisted of 20,294 filtered SNPs across 344 individuals (Table S2). Genetic variation varied greatly across catchments (Table 1): percentage of polymorphic loci from 37% to 79%, H_O from 0.066 to 0.236, and H_E from 0.069 to 0.246, with values being similar among sites within the same catchment. Genetic variation was greatest in the center of the species range, namely, the Cooper, Macumba. and Neales catchments of the Lake Eyre Basin. Estimates of N_e were small and varied from 18 to 519 among catchments, with the lowest in the peripheral Finke River and Murray-Darling Basin populations. F_{IS} was low and nonsignificant ($P > 0.05$) regardless of the sampling site or catchment.

Population structure analyses revealed little to nil differentiation among sites in the same catchment, but substantial differentiation between the eight catchments as well as between basins. Cross-validation error K^*_{CV} in ADMIXTURE indicated

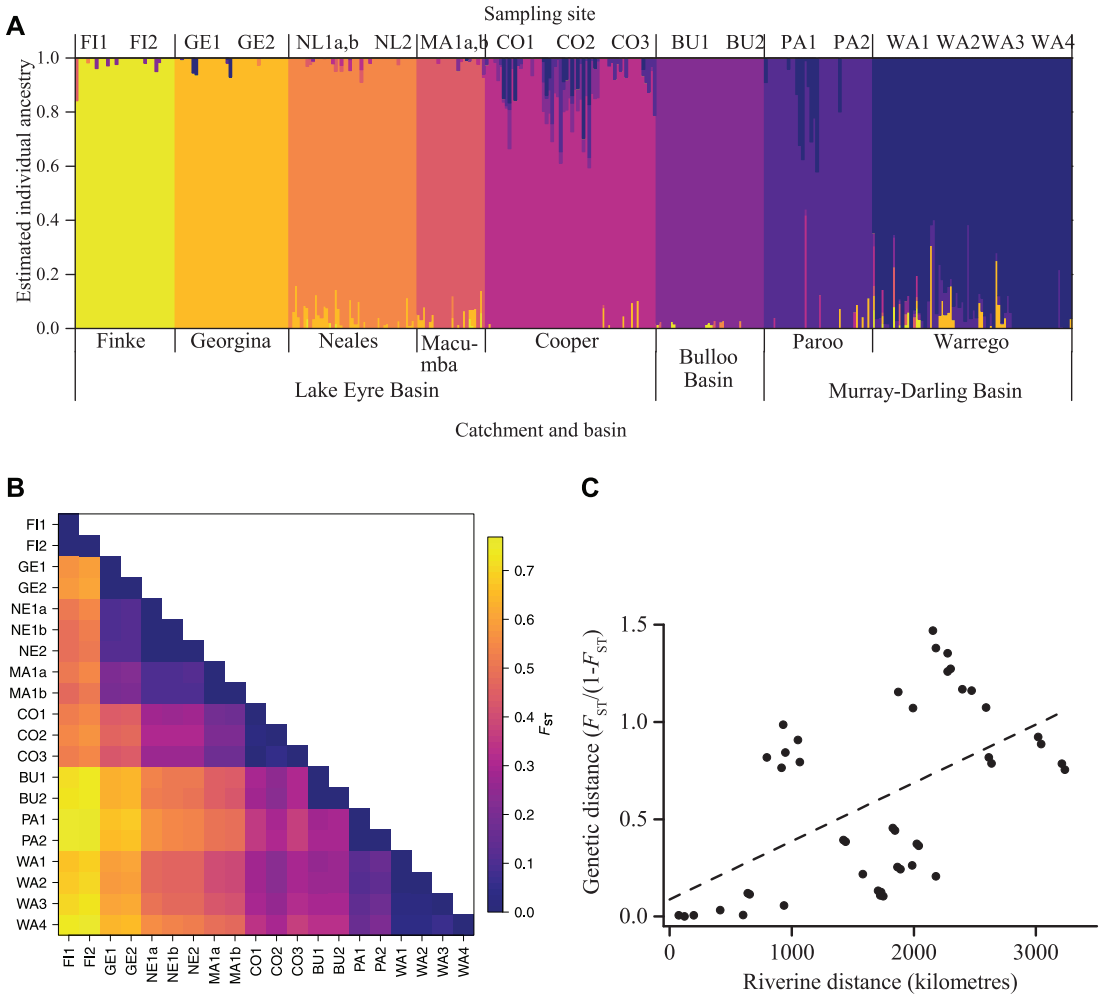

Figure 2. **Neutral population dynamics based on 20,254 SNPs for 351 desert rainbowfish sampled across the geographic and environmental range of the species. Sampling sites are coded following Table 1. (A) Results of ADMIXTURE analysis showing that the most likely number of populations corresponds to the eight sampled catchments ($K = 8$ has the least cross-validation error; Fig. S3). (B) Genetic distance based on F_{ST} heat map among subpopulation samples (exact F_{ST} values and associated *P*-value are in Table S3). (C) Isolation by riverine distance analysis across sampling sites in the Lake Eyre Basin ($r^2 = 0.296$; $P < 0.001$).**

8 as the number of clusters (i.e., K; Fig. S3), which separated the eight catchments (Fig. 2; also see Fig. S4). FastSTRUCTURE produced a K^*_{ϵ} (i.e., the K that has the greatest marginal likelihood) of 6 and $K^*_{\phi c}$ (i.e., the minimal K that best explains ancestry of data) of 8. The latter statistic is expected to better elucidate low levels of structure than the former (Raj et al. 2014). The F_{ST} values ranged from nil to 0.77, with the lowest values among sites in the same catchment (0.000–0.065) and the greatest among catchments ($F_{ST} = 0.096$–0.769) (Fig. 2; Table S3). The F_{ST} values were low between the temporal replicates (Table S3). The AMOVA showed greater genetic variance among catchments than among sites within the same catchment (Table S4). There was significant isolation by distance ($r^2 = 0.296$; $P < 0.001$) in the Lake Eyre Basin (Fig. 2C). The gene flow estimates from BAYESASS3-SNPs among the identified populations (i.e., catchments) were nil to low, with 95% credible intervals almost always encompassing zero and no obvious contribution from any particular catchment into another (Table S5). The mtDNA network showed that haplotypes are genealogically closely related, ruling out the possibility of historically divergent lineages or putative cryptic species among our catchment samples (Fig. S5; also Tables S6 and S7).

ADAPTIVE POPULATION DIVERSITY

The literature search resulted in 31 articles. After manual filtering, only nine of these were population genomic studies on aquatic fauna in arid environments (Martin et al. 2016; Beheregaray et al. 2017; Black et al. 2017; Gates et al. 2017; Attard et al. 2018; Chen et al. 2018a; Chafin et al. 2019; Gouin et al.

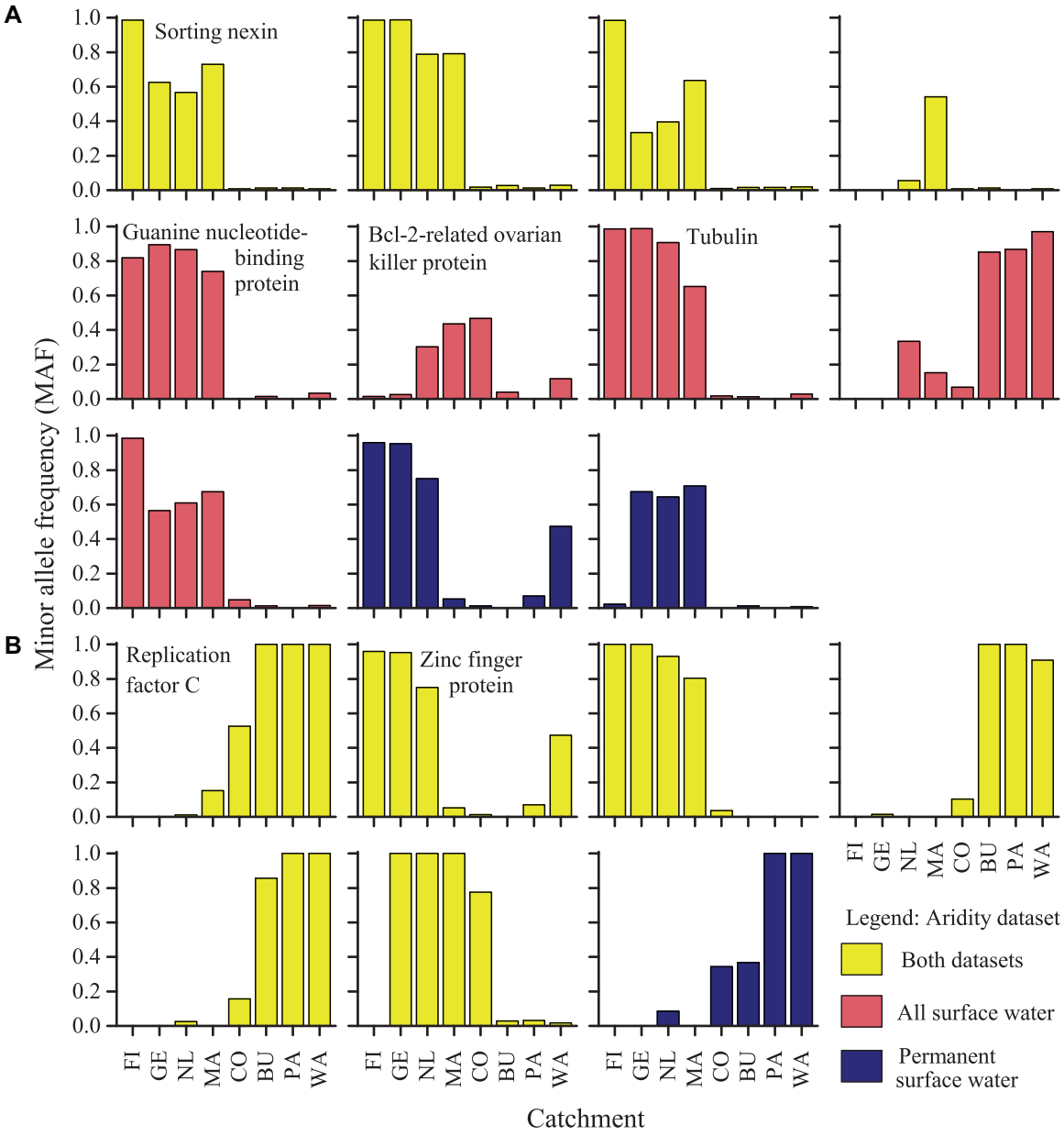

Figure 3. **Allele frequencies at 18 candidate adaptive loci in desert rainbowfish from (A) BAYENV2 and (B) BAYSCENV analyses. Columns of each graph are coloured according to whether the locus had a genotype-environment association using all surface water (permanent and transient), permanent surface water, or both of these datasets. The minor allele is defined as the allele with the lowest frequency across all samples. The protein name is stated for the six annotated candidate SNPs (also see Table S9). Catchments are coded following Table 1.**

2019; Mussmann et al. 2020). Only two looked at adaptation to arid environments (see *Discussion*).

The genotype-environment association methods identified 18 SNPs putatively under divergent selection in desert rainbowfish (Fig. 3). BAYENV2 and BAYSCENV identified 11 and seven of these candidate loci, respectively, with no overlap between methods. Ten of these loci were detected using either of the two aridity indexes (Table S8), with an additional five detected using only the all surface water index and three using the permanent water index. Six of the 18 candidate SNPs were successfully annotated to a protein (Fig. 3; Table S9).

Simulations for the neutral models generated expected patterns of diversity and differentiation based on population size and migration (Figs. S6–S8, left column). In contrast, models that included selection in subpopulation 1 showed increased differentiation and H_E. The scale of this effect on H_E varied depending on the relative balance between drift, migration, and selection. In the $N_e = 100$ scenario (Fig. S6), weak selection produced similar

results to the neutral models, strong selection with low connectivity increased H_E in subpopulation 1 (Fig. S6C), and strong selection with high connectivity increased H_E across the entire metapopulation (Fig. S6L). The $N_e = 500$ scenario (Fig. S7) had similar patterns to $N_e = 100$, but the reduced effect of drift resulted in increased H_E across all models with selection relative to neutral scenarios. H_E was increased globally for all $N_e = 1000$ scenarios that included selection (Fig. S8).

Discussion

The persistence of aquatic populations in desert environments is an enigma that can be better understood through integrating adaptive diversity into more traditional neutral population analyses. We do so here by capitalizing on high-resolution environmental and genome-wide data for a desert fish with a range across central Australia. We found desert rainbowfish move within a catchment likely when hydrological connectivity allows, but hydrological and dispersal limitations inhibit movement between catchments. We found signs of possible adaptive divergence in desert rainbowfish, even among small populations at range edges. This interaction between neutral metapopulation dynamics and aridity-driven putative selection provides insights into the eco-evolutionary processes that maintain adaptive genetic variation and population persistence in extreme environments.

METAPOPULATIONS ALLOW PERSISTENCE IN THE DESERT

All populations of obligate aquatic desert fauna inevitably contract to waterhole or spring refugia during dry periods, and disperse (or not) to different extents when there is hydrological connectivity (Sheldon et al. 2010). The connectivity pattern identified here for desert rainbowfish fits the classic stream hierarchy model, where connectivity is greatest within a catchment but limited between catchments (Hughes and Hillyer 2006; Huey et al. 2008; Mossop et al. 2015). Connectivity within catchments is expected to rely on pulses of flood between metapopulation demes and could be aided by the low topography of central Australia. Conversely, flow between desert catchments is only possible from rare, large flood events. There was also evidence of isolation by riverine distance, yet contemporary gene flow showed no clear geographic pattern between catchments. This is expected because the isolation by distance analysis captures longer term connectivity, compared to the estimates for the last few generations captured in our gene flow analysis.

Desert populations also have the demographic characteristics of small N_e and accompanying strong genetic drift, loss of genetic diversity, and high extinction risk (Frankham et al. 2002). We found that most desert rainbowfish populations have a N_e of only tens to hundreds, which is below the thousand or more theoretically needed for evolutionary resilience (Frankham et al. 2014). There was no population genomic evidence of inbreeding, and so these desert rainbowfish populations probably have random mating. Random mating is potentially promoted by the rainbowfish breeding more during favorable flooding events.

The eastern and western extremes of the species' range often had the lowest estimates of N_e and genetic diversity, which is possibly due to low suitability of edge habitat (Vucetich and Waite 2003) or lower total immigration than the central range. The exception in this pattern was the easternmost catchment (Warrego) that showed moderate diversity. This is possibly due to low levels of introgression from the Murray River rainbowfish (*Melanotaenia fluviatilis*) found in neighboring regions of the Murray-Darling Basin, as introgression can occur among these rainbowfish species (Unmack et al. 2013).

ADAPTIVE DIVERGENCE AND ITS INTERACTION WITH METAPOPULATION DYNAMICS

The adaptive evolution aspect of eco-evolutionary processes likely relies on standing genetic variation (Barrett and Schluter 2008; Hendry 2013). Standing genetic variation in the desert would conceivably undergo strong selection in refugial subpopulations during drought periods. Increased connectivity and population sizes during floods would then ensure retention of adaptive diversity and evolutionary population persistence in the desert. Despite that genome-wide datasets can now readily be used to explore adaptive diversity and adaptive potential in aquatic systems (Grummer et al. 2019), there have only been two such studies of desert aquatic obligates based on our literature search. One study found signs of possible adaptive divergence in golden perch of the Murray-Darling Basin despite high gene flow, and this divergence was associated with the hydroclimatically disturbed arid region of the basin (Attard et al. 2018). The other study was of redband trout in the United States (Chen et al. 2018a), and focused on phenotypic and adaptive divergence between arid and montane environments to extend previous work (Narum et al. 2010; Narum et al. 2013; Chen et al. 2018b). This means we know little about how adaptive evolution interacts with neutral processes in desert environments.

The genotype-environment associations in desert rainbowfish identified candidate adaptive loci associated with aridity. The candidate loci showed similar allele frequencies across the species' range: often one allele approached fixation in the west, and the alternate allele approached fixation in the east (Fig. 3). This is despite that the genotype-environment association analyses detected different candidate loci, likely due to their distinct assumptions (Günther and Coop 2013; de Villemereuil and Gaggiotti 2015). The pattern in allele frequencies aligns with the hydroclimatic gradient from greater aridity and infrequency rainfall in the west (Unmack 2001; Costelloe and Russell 2014), to

more regular seasonal rainfall in the east (Kingsford et al. 2001; McMahon et al. 2008). The signs of possible adaptive divergence hold true even at the edge of the species' range, where there is especially low N_e and genetic diversity.

The most intriguing candidate adaptive marker is a nonsynonymous SNP. This annotated to a guanine nucleotide-binding protein (G protein), which are involved in transduction of extracellular to intracellular signals (Syrovatkina et al. 2016). G proteins are used in fish to detect taste, smell, and salinity (Nearing et al. 2002; Oka and Korsching 2011; Gao et al. 2017), to control light sensitivity for vision (Strickler and Jeffery 2009; Gross et al. 2013), and in sensors that detect water flow (Chitnis et al. 2012). The nonsynonymous substitution likely did not undergo negative selection because the amino acids involved hold similar biochemical properties. The resulting potential changes in environmental sensing may increase fitness in the high environmental stochasticity of the desert (Costelloe et al. 2005; Sheldon and Fellows 2010; Preite and Pearson 2017).

The remaining annotated candidate loci are involved in biological functions potentially linked to extreme environments. Replication factor C is involved in DNA replication and repair (Mossi and Hübscher 1998), which is also the function of another candidate locus identified in golden perch for adaptation to aridity (Attard et al. 2018). Candidate loci also had functions related to apoptosis, or controlled cell death, a common response to stressful environmental conditions (reviewed for fish in AnvariFar et al. 2017). The annotated locus with the most direct connection to cell death is Bcl-2-related ovarian killer protein (BOK), which is part of the B-cell lymphoma (Bcl-2) gene family that controls cell survival and apoptosis (Tsujimoto and Shimizu 2000; Kratz et al. 2006; Youle and Strasser 2008; Moldoveanu et al. 2014). BOK is mostly known for its high expression in female reproductive tissue (Hsu et al. 1997; Hsu and Hsueh 2000; Böhne et al. 2014), and may play a role in reabsorption of unspawned eggs during, for example, unfavorable environmental conditions (Morais et al. 2012; Ke et al. 2013; Morais et al. 2016).

The eco-evolutionary simulations demonstrate how the balance between selection, population size, and connectivity can modulate the maintenance of genetic diversity in metapopulations. The simulations spanned a realistic (but intentionally simplified) range of parameters based on the empirical results. They suggest that genomic regions linked to loci involved in local adaptation can increase divergence among subpopulations, while periodic migration then generates higher levels of genetic diversity than generally expected for small, isolated populations. Even when adaptive alleles are lost due to drift, they may be maintained elsewhere in the metapopulation through selection, and spread by migration during floods. These processes shape diversity in the local subpopulations subject to selection and, with sufficient migration, across the whole metapopulation. The simulations also suggested that the effective migration rate over the long term is more important in shaping the distribution of diversity across the metapopulation than the specific flood regime that facilitates migration. Together, results from the genotype-environment association analyses and the eco-evolutionary modeling suggest a possible evolutionary mechanism promoting the maintenance of adaptive diversity in desert fish metapopulations: strong selection occurs during long periods of isolation within refugial subpopulations, before diversity is redistributed among demes during flood periods.

NEXT STEPS

Desert rainbowfish can be brought from the field and into the lab to provide experimental insights into the signal of possible adaptive divergence detected here, in line with experiments in other rainbowfishes (McGuigan et al. 2003; Smith et al. 2013; McCairns et al. 2016; Sandoval-Castillo et al. 2020). Studies on desert rainbowfish based on transcriptomes (e.g., Sandoval-Castillo et al. 2020) and whole genomes can provide higher resolution records of variants and detailed information about causative genes. Such studies will allow better prediction and management of climate impacts on species (Bay et al. 2018). The role of metapopulation dynamics in maintaining alleles from adaptively divergent populations, such as desert rainbowfish refugia, could aid the persistence of species in future climates (Bell and Gonzalez 2011; Attard et al. 2018). We advocate studying adaptive diversity in extreme environments and its connections to traditional population demographics to better understand eco-evolutionary dynamics and manage the impacts of climate change.

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AUTHOR CONTRIBUTIONS

LBB conceived the study. CRMA generated the data with contributions from all other authors. CRMA analyzed the data with contributions from JS-C and LBB. CRMA drafted the article together with LBB. CJB designed, ran, and wrote about the simulations with LBB. All authors contributed to data interpretation and critically revised the article.

DATA ARCHIVING

Data are available through FigShare at https://figshare.com/search?q=10.6084%2Fm9.figshare.16915669.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Simulation parameters. N_e = subpopulation size; S = fitness advantage for alternate allele of each locus under selection (0 = neutral); M = migration rate during flood periods (no migration during dry periods); C = flood frequency (generations); M_e = effective number of migrants received per generation.

Table S2 Number of SNPs retained after each filtering step in desert rainbowfish.

Table S3 F_{ST} matrix for desert rainbowfish at 20,254 SNPs. Site codes are following Table 1. F_{ST} values are in the lower diagonal, and P-values in the upper diagonal. Shaded gray are comparisons between sites within the same catchment.

Table S4 Analysis of molecular variance (AMOVA) of desert rainbowfish at 20,254 SNPs. Temporal samples from the same site were combined into one site for this analysis.

Table S5 Migration rate estimates from BAYESASS3-SNPs between each population (i.e., catchment) of desert rainbowfish using 20,254 SNPs.

Table S6 Frequencies of mtDNA cytochrome *b* haplotypes depicted in Figure S5 haplotype network. *n*, sample size.

Table S7 Sequences for each mtDNA cytochrome *b* haplotype (275 bp fragment) in Figure S5 haplotype network.

Table S8 Aridity indices for genotype-environment association analyses. Permanent water refers to the amount of area (km^2) with permanent surface water, defined as water observed at least 80% of the time. Permanent and transient water refers to the amount of area (km^2) equivalent to water observed 100% of the time when water observations are amalgamated over the entire catchment. Data are extracted from Water Observations from Space (http://www.ga.gov.au/scientific-topics/hazards/flood/wofs). These raw values were standardized before analyses.

Table S9 Annotations of candidate SNPs in desert rainbowfish to UniProt database, and their scaffold and position on the rainbowfish reference genome. Only hits with an *e*-value of at least 1×10^{-2} were considered within 500 bp of the SNP. When there were multiple hits for one locus, the hit with the lowest *e*-value was retained.

Figure S1 Schematic representation of the eco-evolutionary simulations used to model boom-bust cycles of population expansion and contraction due to flooding. The shaded area represents a flood event where subpopulation sizes increase by 10× before declining by 1/3 each generation until returning to baseline refugial population sizes. During flood events, migration could occur between all subpopulations. Three subpopulation sizes ($n = 100$, 500, and 1000) were simulated to examine a range of migration rates, flooding frequencies (x), and selection strengths. For simulations that included selection, selection only occurred in refugial habitat during dry periods, with all loci considered neutral during flood events. Cycles repeated for a total duration of 20,000 generations for all simulations.

Figure S2 Burn-in period of 100,000 generations simulated based on an island model with three subpopulations of 5000 individuals each with a migration rate of 0.01. Each metapopulation scenario was initiated at generation 100,001 following from this model, assuming population 1 as the ancestral population.

Figure S3 Cross-validation error from ADMIXTURE for desert rainbowfish at 20,254 SNPs. The cross-validation error was lowest for $K = 8$, but as it was similarly low for $K = 9$ and 10, the ancestry proportions of these K were also visualized (Fig. S4).

Figure S4 Clustering results of ADMIXTURE analysis for desert rainbowfish using 20,254 SNPs at K of (a) 9 and (b) 10. This reveals substructure within Cooper (black) and Warrego (white). Sampling sites are coded following Table 1.

Figure S5 Haplotype network of mtDNA cytochrome *b* for desert rainbowfish. Each circle represents a haplotype, with size corresponding to its observed frequency; each line represents a single nucleotide difference; ovals represent unsampled or extinct haplotypes. The data used to create the network are in Tables S6 and S7.

Figure S6 Simulations for three refugial subpopulations of 100 individuals. (A–C) Flood events every 500 generations where population sizes increased to 1000, before declining by 1/3 each generation until returning to 100. Migration rate of 0.01 during floods. (D–F) Flood events every 100 generations where population sizes increased to 1000, before declining by 1/3 each generation until returning to 100. Migration rate of 0.01 during floods. (G–I) Flood events every 500 generations where population sizes increased to 1000, before declining by 1/3 each generation until returning to 100. Migration rate of 0.05 during floods. (J–L) Flood events every 100 generations where population sizes increased to 1000, before declining by 1/3 each generation until returning to 100. Migration rate of 0.05 during floods. In each case, there was no migration during dry periods. The first column are neutral simulations with no selection. Columns two and three model natural selection where 2.5% of loci provide a fitness advantage of 0.001 and 0.01 in subpopulation 1. During flood events all loci are neutral. Shaded areas represent 95% CIs, based on 100 replicate simulations.

Figure S7 Simulations for three refugial subpopulations of 500 individuals. (A–C) Flood events every 500 generations where population sizes increased to 5000, before declining by 1/3 each generation until returning to 500. Migration rate of 0.01 during floods. (D–F) Flood events every 100 generations where population sizes increased to 5000, before declining by 1/3 each generation until returning to 500. Migration rate of 0.01 during floods. (G–I) Flood events every 500 generations where population sizes increased to 5000, before declining by 1/3 each generation until returning to 500. Migration rate of 0.05 during floods. (J–L) Flood events every 100 generations where population sizes increased to 5000, before declining by 1/3 each generation until returning to 500. Migration rate of 0.05 during floods. In each case there was no migration during dry periods. The first column are neutral simulations with no selection. Columns two and three model natural selection where 2.5% of loci provide a fitness advantage of 0.001 and 0.01 in subpopulation 1. During flood events all loci are neutral. Shaded areas represent 95% CIs, based on 100 replicate simulations.

Figure S8 Simulations for three refugial subpopulations of 1000 individuals. (A–C) Flood events every 500 generations where population sizes increased to 10,000, before declining by 1/3 each generation until returning to 1000. Migration rate of 0.01 during floods. (D–F) Flood events every 100 generations where population sizes increased to 10,000, before declining by 1/3 each generation until returning to 1000. Migration rate of 0.01 during floods. (G–I) Flood events every 500 generations where population sizes increased to 10,000, before declining by 1/3 each generation until returning to 1000. Migration rate of 0.05 during floods. (J–L) Flood events every 100 generations where population sizes increased to 10,000, before declining by 1/3 each generation until returning to 1000. Migration rate of 0.05 during floods. In each case there was no migration during dry periods. The first column are neutral simulations with no selection. Columns two and three model natural selection where 2.5% of loci provide a fitness advantage of 0.001 and 0.01 in subpopulation 1. During flood events all loci are neutral. Shaded areas represent 95% CIs, based on 100 replicate simulations.