

## Phylogeographic structure, demographic history and morph composition in a colour polymorphic lizard

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### Abstract

In polymorphic species, population divergence in morph composition and frequency has the potential to promote speciation. We assessed the relationship between geographic variation in male throat colour polymorphism and phylogeographic structure in the tawny dragon lizard, *Ctenophorus decresii*. We identified four genetically distinct lineages, corresponding to two polymorphic lineages in the Northern Flinders Ranges and Southern Flinders Ranges/Olary Ranges regions respectively, and a monomorphic lineage in the Mt Lofty Ranges/Kangaroo Island region. The degree of divergence between these three lineages was consistent with isolation to multiple refugia during Pleistocene glacial cycles, whereas a fourth, deeply divergent (at the interspecific level) and monomorphic lineage was restricted to western New South Wales. The same four morphs occurred in both polymorphic lineages, although populations exhibited considerable variation in the frequency of morphs. By contrast, male throat coloration in the monomorphic lineages differed from each other and from the polymorphic lineages. Our results suggest that colour polymorphism has evolved once in the *C. decresii* species complex, with subsequent loss of polymorphism in the Mt Lofty Ranges/Kangaroo Island lineage. However, an equally parsimonious scenario, that polymorphism arose independently twice within *C. decresii*, could not be ruled out. We also detected evidence of a narrow contact zone with limited genotypic admixture between the polymorphic Olary Ranges and monomorphic Mt Lofty Ranges regions, yet no individuals of intermediate colour phenotype. Such genetic divergence and evidence for barriers to gene flow between lineages suggest incipient speciation between populations that differ in morph composition.

### Introduction

Spatially structured phenotypic variation within species generally reflects adaptation to local selective pressures coupled with reduced gene flow and is a common precursor to speciation (Endler, 1977). The degree of phenotypic variation within populations can also vary geographically. The clearest example is geographic variation in polymorphism, whereby populations differ in the number, type and frequency of coexisting discrete

phenotypes (morphs; McLean & Stuart-Fox, 2014). Polymorphic species may be more likely to be geographically variable because phenotypic variation permits utilization of a wider range of habitats and large range sizes while decreasing susceptibility to environmental change (Forsman *et al.*, 2008). Furthermore, processes generating geographic variation in morph composition are also likely to promote divergence between populations (e.g. Corl *et al.*, 2010; Iserbyt *et al.*, 2010). Consequently, geographic variation in polymorphism may facilitate and be an important precursor to speciation (West-Eberhard, 1986; Hugall & Stuart-Fox, 2012; McLean & Stuart-Fox, 2014).

Recent research has increased our understanding of the potential role of polymorphism in diversification

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(Gray & McKinnon, 2007; Forsman *et al.*, 2008; Corl *et al.*, 2010; McKinnon & Pierotti, 2010; Hugall & Stuart-Fox, 2012); however, most research has focused on within population processes, and relatively few studies have examined geographic variation in polymorphism, with specific focus on its causes and/or consequences (reviewed in McLean & Stuart-Fox, 2014). For polymorphism to be maintained within a population, morphs must have an equal fitness over time (Maynard-Smith, 1966). Often, morphs differ not only in appearance but also in aspects of behaviour, morphology and physiology (McKinnon & Pierotti, 2010). These trait complexes represent alternative fitness optima and allow multiple morphs to persist in a population (Sinervo & Lively, 1996). Changes in the number or frequency of morphs present within a population can alter competitive interactions and affect population dynamics, potentially leading to the development of new trait complexes (Sinervo & Svensson, 2002). Therefore, populations that differ in morph composition may become incompatible, either genetically or through divergent mate preferences for particular traits. Understanding the geographical context of polymorphism is thus important for understanding the role of polymorphism in speciation.

Geographic patterns of variation in morph composition and frequencies are likely shaped by the complex interactions between multiple evolutionary processes, including local adaptation, frequency-dependent selection, genetic drift and gene flow (reviewed in McLean & Stuart-Fox, 2014). Nonadaptive stochastic and historical events may leave a detectable genetic signature (Eckert *et al.*, 2008); for example, morphs may be lost by chance during population contraction and fragmentation in response to glacial cycles. Where this occurs, we expect populations with fewer morphs to have low genetic diversity due to historical bottlenecks. Conversely, the maintenance of a high degree of polymorphism is expected to be associated with relatively large and stable population size, and hence high genetic diversity. Alternatively, genetic diversity may reflect the nature of selection in populations. Polymorphism is often maintained by correlational and frequency-dependent selection working on a suite of co-adapted traits (Sinervo *et al.*, 2000; Sinervo & Clobert, 2003; Svensson *et al.*, 2005), and such balancing selection can maintain or increase genetic diversity (Clarke, 1979; Charlesworth, 2006). By contrast, populations that become fixed for a single morph can exhibit rapid trait evolution (Corl *et al.*, 2010), and the strong directional selection required for such rapid evolution will deplete genetic variation (Maynard-Smith & Haigh, 1974). Therefore, polymorphic populations may have higher genetic diversity than monomorphic populations because morph changes can fundamentally alter the strength and nature of selection. Consequently, detailed phylogeographic and population

genetic studies are essential to identify potential processes generating geographic variation in polymorphism and facilitating genetic divergence between populations.

The Australian *Ctenophorus decresii* species complex is a monophyletic group comprising four closely related agamid lizards: *C. decresii*, *Ctenophorus fionni*, *Ctenophorus tjantjalka* and *Ctenophorus vadhappa* (Houston & Hutchinson, 1998; Melville *et al.*, 2001; Chen *et al.*, 2012). Intraspecific colour variation is ubiquitous in this group (Houston, 1974; Johnston, 1992). Firstly, all species are sexually dimorphic with cryptic females and larger, brightly coloured males which perform conspicuous behavioural displays during courtship and territory defence (Gibbons, 1979). Furthermore, male coloration is the main feature distinguishing species (Houston, 1974). Secondly, there is substantial geographic colour variation resulting in multiple distinct regional colour forms (Houston, 1974). These colour forms differ in the extent of local adaptation to differently coloured backgrounds, particularly in males, and body regions that vary most among populations are also the most sexually dichromatic (Stuart-Fox *et al.*, 2004). Thus, the marked geographic variation in male coloration in this species group is likely to reflect both local adaptation and selection for signalling functions, including species recognition where species ranges abut or overlap (Stuart-Fox, 2003; Stuart-Fox *et al.*, 2004).

Colour variation is particularly striking in the tawny dragon lizard, *C. decresii* (Duméril & Bibron, 1837). Male *C. decresii* are polymorphic for throat coloration (Teasdale *et al.*, 2013); however, the geographical context of the polymorphism has yet to be documented. At least two regional colour forms, corresponding to a northern and southern 'race', have been identified in the species (Houston, 1974); however, it is currently unknown how this colour variation corresponds to genetic variation and degree of polymorphism. In this study, we comprehensively survey eleven populations across the range of *C. decresii* to quantify geographic variation in the number and frequency of male throat colour morphs. Using multilocus phylogeographic data, incorporating the full distribution of *C. decresii*, we investigate how biogeographic and demographic histories have shaped the current distribution of colour variation in the species. Under a scenario of morph loss during historical bottlenecks, we expect signatures of population contraction, and lower genetic diversity, in populations with fewer morphs. Furthermore, we test whether population divergence in morph composition is associated with genetic divergence and reduced levels of gene flow. Our study provides insight into how colour variation, and specifically geographic variation in polymorphism, relates to genetic divergence among populations and, potentially, incipient speciation.

## Materials and methods

### Study system

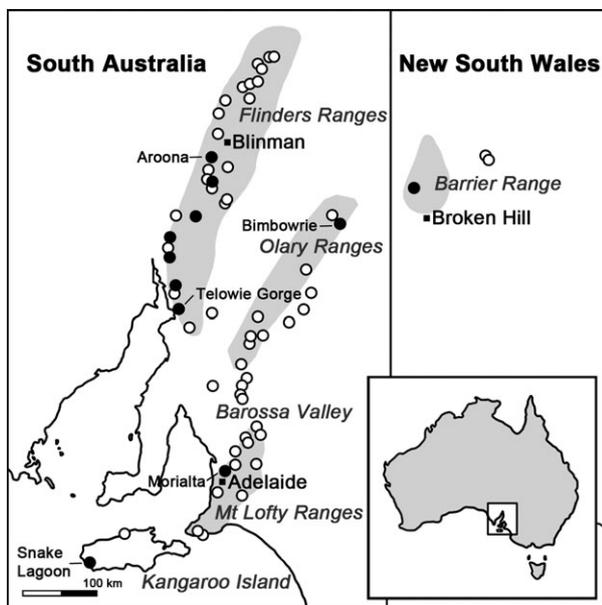
*Ctenophorus decresii* is a small ( $\leq 30$  g) rock-dwelling agamid lizard distributed throughout eastern South Australia (SA) and far western New South Wales (NSW; Fig. 1). Although restricted to rocky outcrops, the species occupies variable habitats ranging from temperate woodland in the south to arid grassland in the north. *C. decresii* is locally abundant in suitable habitat; however, it is listed as endangered in NSW where it is currently known from only two populations (Sass & Swan, 2010). Based on phylogeographic structure (Chapple *et al.*, 2005; Smith *et al.*, 2007; McCallum *et al.*, 2013), and phenotypic divergence (Ford, 1987; Chapple *et al.*, 2008; Siström *et al.*, 2012) detected in other species, we recognized *a priori* six geographic regions corresponding to the Northern Flinders Ranges, Southern Flinders Ranges, Olary Ranges, Mt Lofty Ranges, Kangaroo Island and western NSW (Fig. 1), which guided survey effort and design.

In *C. decresii*, male throat coloration develops at sexual maturity and is fixed for life (as observed in captive lizards and mark-recapture surveys, Osborne, 2004; M. Yewers & D. Stuart-Fox, unpublished data), and does not vary with body size, such that all morphs are observed in the same age/size class (Teasdale *et al.*, 2013; K. Rankin & D. Stuart-Fox, unpublished data). To determine colour variation within and among populations, we conducted field surveys across the species

range (Fig. 1) during late spring and summer between 2010 and 2012.

Previous work on two populations of *C. decresii* in the Southern Flinders Ranges used digital colour pattern analysis, spectrophotometry and discriminant function analysis (DFA) to demonstrate the presence of discrete male throat colour morphs (Teasdale *et al.*, 2013). Teasdale *et al.* (2013) showed that males can be reliably categorized into four morphs: pure orange, pure yellow, orange and yellow combined, and grey (which lacks both orange and yellow; Teasdale *et al.*, 2013), based on the presence or absence of orange and yellow. Using the same standardized photography and the above criteria, we visually assessed throat colour polymorphism for a total of 281 adult males across 11 populations (Fig. 1). We photographed lizards using a Canon PowerShot SX1-IS digital camera (saved in RAW format; Stevens *et al.*, 2007), and each photograph included a ruler and a grey card (Miconova). Photographs were then linearized for radiance and calibrated (relative to the grey card) to account for differences in illumination between photos (Stevens *et al.*, 2007). Within each population, we surveyed 20–30 males, which was a large enough sample size to detect rare morphs at a relative frequency of approximately 5%.

Given that throat coloration has not been previously quantified for populations from the Mt Lofty Ranges, Kangaroo Island and western NSW, we used digital colour pattern analysis to assess colour pattern variation for males from these regions. Photographs were rescaled relative to the smallest image and just the throat was cut out and transferred to a constant sized white background. We performed a segmentation analysis on the standardized image which calculates the proportion of orange, yellow, grey and blue for each individual using the RGB values for each pixel on the throat (Teasdale *et al.*, 2013). Based on preliminary analysis, we applied colour threshold values of 0.35, 0.18 and 0.02 for red, yellow and blue, respectively. Images were then converted to greyscale, and a 'granularity' pattern analysis was performed to determine the predominant marking size and overall pattern contrast (Spottiswood & Stevens, 2010; Stoddard & Stevens, 2010). Image standardisation and colour and pattern analyses were performed using custom written programs in MATLAB (The MathWorks Inc., Natick, MA, USA; Teasdale *et al.*, 2013). As morph categories could not be easily predefined for these populations, we performed a principle component analysis (PCA; rather than a DFA which requires predefined categories) incorporating the proportion of orange, yellow, grey and blue and a measure of overall patterning (total power) using the FactoMineR package in R (Lê *et al.*, 2008; R Development Core Team, 2010) and examined the clustering of individuals based on the axes of the first two principal components. Lizards which were molting were excluded from the analysis.



**Fig. 1** Distribution of the tawny dragon lizard, *Ctenophorus decresii*. Each circle represents a site for which tissue was sampled with black circles indicating sites also extensively surveyed to assess coloration. Elevated rocky ranges are highlighted in grey.

We were unable to analyse coloration in the Northern Flinders Ranges (north of Blinman, SA; Fig. 1) due to a recent reduction of *C. decresii* numbers in this region; however, we examined all available museum specimens. Although orange and yellow coloration fades considerably on spirit-preserved specimens, we could detect subtle differences in colour and pattern between individuals. All tissues sampled from the Northern Flinders Ranges were historical with the most recent specimens recorded in 1999. In this region, *C. decresii* overlaps in distribution with the closely related *C. vadrappa*, which is endemic to the Northern Flinders Ranges. Although the species have been recorded in sympatry (Gibbons & Lillywhite, 1981) and in similar densities (Telfer, 2000) in the past, *C. vadrappa* currently dominates the Northern Flinders Ranges, whereas we did not find *C. decresii* during intensive surveys in 2011 and 2012 of all previously recorded localities. Consequently, *C. decresii* may now be absent or present in very low numbers in the Northern Flinders Ranges.

### Tissue and sequence data collection

We collected *C. decresii* tissue samples from localities in SA and NSW (Fig. 1). Tissues were sampled nondestructively (10 mm tail clip) and stored in 99% ethanol for subsequent molecular analysis. To ensure that individuals from across the species entire range were included in the analysis, we also subsampled museum specimens from the South Australian Museum (Adelaide) and Australian Museum (Sydney). A total of 260 *C. decresii* samples were included in this study and used for either sequence or microsatellite data or both (Table S1).

Genomic DNA was extracted from tissue with proteinase-K and a GenCatch (TM) Blood & Tissue Genomic Mini-Prep Kit (Epoch Life Sciences, Sugar Land, TX, USA). We selected 110 *C. decresii* samples for mitochondrial (mtDNA) analysis and amplified an 850-base pair region of the mitochondrial genome including the NADH dehydrogenase subunit four (ND4), tRNA<sup>his</sup>, tRNA<sup>ser</sup> and tRNA<sup>leu</sup> regions using the primers ND4F (Forstner *et al.*, 1995) and tRNA-Leu (Scott & Keogh, 2000; Driscoll & Hardy, 2005). Where individual samples would not amplify, we used an alternate forward primer, ND4F3 (Driscoll & Hardy, 2005), and trimmed the resulting sequence to size. A subset of 57 samples, encompassing the full geographic range and mtDNA diversity of *C. decresii*, were selected for sequencing of five nuclear loci. Published primers were used to amplify the  $\alpha$ -enolase, intron 8 (Melville *et al.*, 2008), BACH1, FSHR, MKL1 (Townsend *et al.*, 2008) and SLC8A1 (Townsend *et al.*, 2011) gene regions. For comparison of intra- and interspecific genetic diversity, we also sequenced four *C. fionni*, two *C. tjantjalka* and nine *C. vadrappa* samples. Additionally, we included *Ctenophorus pictus* and *Ctenophorus rufescens* as out-group species in the analyses (Table S1).

All amplification reactions were 25  $\mu$ L in total volume, containing approximately 20 ng template DNA, 12.5  $\mu$ L GoTaq Hot Start Master Mix (Qiagen, Hilden, Germany) and 0.4  $\mu$ M forward and reverse primer. Cycling conditions varied among loci but broadly consisted of an initial denaturing step at 95 °C for 5 min followed by 35 cycles of 94 °C for 45 s, annealing temperature for 45 s, and 72 °C for 1.5 min, with a final extension step at 72 °C for 10 min. The PCR product was sent to Macrogen (Korea) for purification and forward and reverse sequencing. Sequences obtained for each individual and gene were aligned, manually edited and assembled into a single consensus sequence using BioEdit ver. 7.0.4.1 (Hall, 1999). Combined we amplified a total of approximately 5282 base pairs across six loci.

### Microsatellite genotyping

We developed microsatellite loci specifically for *C. decresii*. DNA enrichment, library development (via 454 sequencing) and primer design were performed by the Savannah River Ecology Laboratory at The University of Georgia (USA). Six polymorphic loci were found to amplify reliably (Ctde03, Ctde05, Ctde08, Ctde12, Ctde21 and Ctde45; Table S2) and were therefore used in this study. An additional four microsatellite loci were amplified using published primers developed for other agamid lizards (AM41, CP10, CP11, and CP17; Schwartz *et al.*, 2007). All loci were checked for the presence of null alleles using Microchecker ver. 2.2.3 (Van Oosterhout *et al.*, 2004) and tested for linkage disequilibria using GenePop ver. 4.2 (Raymond & Rousset, 1995; Rousset, 2008). We calculated levels of heterozygosity and Hardy–Weinberg equilibria using Arlequin ver. 3.5 (Excoffier *et al.*, 2005). In total, we genotyped 10 microsatellite loci for 230 individuals (Table S1).

For most microsatellite loci, amplification reactions were 20  $\mu$ L in total volume, containing 10  $\mu$ L GoTaq Hot Start Master Mix (Qiagen), 0.4  $\mu$ M forward and reverse primer and approximately 20 ng template DNA. Remaining loci were amplified in 12.5  $\mu$ L reactions containing 2.5 units HotStarTaq (Qiagen), 0.4  $\mu$ M forward and reverse primer, 1 $\times$  PCR buffer (containing Tris-Cl, KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>; pH 8.7), 1 $\times$  Q solution, 250  $\mu$ M dNTP, 3.0 mM MgCl<sub>2</sub> and approximately 20 ng template DNA. Thermal cycling conditions consisted of an initial denaturing step at 95 °C for 5 min (15 min for HotStarTaq) followed by 42 cycles of 94 °C for 30 s, annealing temperature (Table S2) for 30 s, and 72 °C for 45 s followed by a final extension step at 72 °C for 10 min. Touchdown cycling parameters consisted of annealing temperatures of 60 °C for two cycles, 55 °C for two cycles, 50 °C for two cycles and 45 °C for 36 cycles. PCR products were sent to Macrogen (Korea) for fragment visualization, and fragment sizes were called using Peak Scanner ver. 1.0 (Applied Biosystems, Foster City, CA, USA).

### Phylogenetic and phylogeographic structure

We implemented MrModelTest ver. 2.3 (Nylander, 2004) to determine the most appropriate models of sequence evolution for our data using the Akaike information criterion (AIC). Bayesian phylogenetic analyses were performed using MrBayes ver. 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For the combined analysis, total sequence data for 57 *C. decresii* and 18 out-group individuals, for which all gene regions were sequenced, were concatenated and partitioned into mtDNA and nuDNA with the partitions parameterised separately. Models selected for the mtDNA and nuDNA were the Generalised Time-Reversible plus gamma (GTR + G) and the Hasegawa, Kishino and Yano (HKY) models, respectively. To investigate the phylogenetic signature from different markers, we also analysed each gene region independently and the five nuclear loci combined and partitioned by gene (Fig. S2). In all cases, four Markov Chain Monte Carlo (MCMC) analyses were run for  $2 \times 10^7$  generations, sampling every 1000 generations with the first 25% of trees discarded as burn-in. Convergence between runs was determined via examination of the standard deviation of split frequencies ( $< 0.01$  indicating convergence).

Using the data set for which all gene regions were sequenced, we employed the multilocus coalescent model implemented in \*BEAST (Heled & Drummond, 2010), an extension of BEAST ver. 1.7.4 (Drummond & Rambaut, 2007), and compared results to those obtained from the concatenated data set. Nuclear sequences were haplotyped using PHASE, ver. 2.1 (Stephens *et al.*, 2001). We converted the nuDNA sequence alignments to the format required by PHASE using SeqPHASE (Flot, 2010). For the \*BEAST analysis, 'species' (populations) were defined using predetermined, reciprocally monophyletic mtDNA lineages (Fig. S2). These were the New South Wales (NSW), Northern Flinders Ranges (NFR), combined Southern Flinders and Olary Ranges (SFR/OR), and combined Mt Lofty Ranges and Kangaroo Island (LR/KI) geographic regions within *C. decresii* and *C. fionni*, *C. rufescens*, *C. tjantjalka*, *C. vadrappa* and *C. pictus*. We performed two independent runs of  $10^8$  generations, sampling every  $10^4$  generations. Models applied were GTR + G for ND4 and HKY for each of the five nuclear genes. The Yule tree prior was used, as our data set encompassed interspecific variation in addition to divergent intraspecific lineages. Based on preliminary runs and an appraisal of the ucd.stder posterior, we used the lognormal relaxed clock model for ND4 and the strict clock model for each of the nuclear genes ( $\alpha$ -enolase, BACH1, FSHR, MKL1, and SLC8A1). Convergence was assessed by comparing posterior probabilities of all parameter estimates and by calculating the effective sample size (ESS) of the combined runs using Tracer ver. 1.5 (Rambaut &

Drummond, 2009). Furthermore, we confirmed that runs were converging on the same tree topology using AWTY (Nylander *et al.*, 2008). We discarded the initial 10% of trees as burn-in and combined the two runs to produce a species tree.

To investigate phylogeographic structure, we calculated net sequence divergence ( $d_A$  using the Tamura-Nei model) between regions for the mtDNA and nuDNA data sets in MEGA ver. 5.1 (Tamura *et al.*, 2011). We performed two separate analyses, one using microsatellite loci and the other using phased nuDNA haplotypes, to assign individuals to populations using the Bayesian clustering program STRUCTURE ver. 2.3.3 (Pritchard *et al.*, 2000). Based on observed mtDNA phylogeographic structure, we ran ten iterations for values of  $K$  ranging from one to six. We used the admixture model, independent allele frequencies, a burn-in length of  $10^6$  steps and MCMC length of  $3 \times 10^6$  steps. The most likely value of  $K$  was determined using the method of Evanno *et al.* (2005) employed in Structure Harvester. We used the program DISTRUCT ver. 1.1 (Rosenburg, 2004) to plot the STRUCTURE output.

### Demographic history

Phylogenetic analysis revealed *C. decresii* to be polyphyletic with NSW populations clearly comprising a separate species. This was confirmed by the STRUCTURE analysis which showed no genetic admixture (see Results). Consequently, we excluded the NSW region from demographic analyses of populations of *C. decresii*. Haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) were calculated in DnaSP ver. 5.10.01 (Librado & Rozas, 2009) based on the full mtDNA data set. Analysis of molecular variance ( $AMOVA$ ; Excoffier *et al.*, 1992) was used to test for genetic differentiation between the remaining five recognized regions using mtDNA and nuDNA data sets in Arlequin ver. 3.5 (Excoffier *et al.*, 2005) based on  $10^5$  simulations. For the microsatellite data set, we calculated global  $F_{ST}$  with and without the exclude null alleles (ENA) correction, which corrects for positive bias due to the presence of null alleles, using the program FreeNA (Chapuis & Estoup, 2007). As mtDNA has a higher mutation rate, and a smaller effective population size than nuDNA, we expect larger  $F_{ST}$  from mtDNA than nuDNA sequence data. Additionally,  $F_{ST}$  from multiple, highly variable loci, such as microsatellite markers, are expected to be small as population differentiation cannot exceed marker homozygosity (Hedrick, 1999). Furthermore, constraints on allele size range and high mutation rate can produce corresponding sets of alleles in divergent populations (Nauta & Weissing, 1996; Selkoe & Toonen, 2006). Consequently, we expect substantially different  $F_{ST}$  from different markers and do not make direct comparison of  $F_{ST}$  between markers.

Microsatellite data were not appropriate to examine demographics at the regional level as the majority of loci showed departures from Hardy–Weinberg equilibrium, primarily driven by subregional genetic structure (see Selkoe & Toonen, 2006). For demographic analysis of microsatellite data, we therefore delineated populations at a finer spatial scale to best meet the assumption of panmixia. We focused on two well-sampled populations in the Southern Flinders Ranges (Aroona and Telowie Gorge) and one in each of the Olary Ranges (Bimbowrie), the Mt Lofty Ranges (Morialta) and Kangaroo Island (Snake Lagoon). Microsatellite loci performed well at this level for populations from the northern range of the species (Aroona, Telowie Gorge, Bimbowrie; Table S3), indicating panmixia. However, for Morialta and Snake Lagoon, multiple loci still showed departures from Hardy–Weinberg equilibrium, and given the finer spatial scale, this would suggest the presence of null alleles. This is likely because microsatellite loci were developed mainly from samples in the Flinders Ranges and screened across only a few samples from the southern range of the species. We therefore performed demographic analyses using both the full microsatellite data set (10 loci) and a reduced data set using only loci in Hardy–Weinberg equilibrium within each population.

For microsatellite data, we calculated observed heterozygosity ( $H_O$ ) and unbiased expected heterozygosity ( $H_E$ ) in Arlequin ver. 3.5 (Excoffier *et al.*, 2005). The program HP-RARE (Kalinowski, 2005) was used to calculate allelic richness ( $A$ ) using the rarefaction correction for unequal sample sizes. Differences in genetic diversity among regions and populations were tested using one-way ANOVAS and Tukey's *post hoc* tests performed in R ver. 3.0.3 (R Development Core Team). We explored the demographic history of populations using a coalescent-based Bayesian analysis as implemented in MSVAR ver. 1.3 (Beaumont, 1999; Storz & Beaumont, 2002). The method assumes that microsatellite loci evolve under the stepwise mutation model (SMM), where a single repeat unit is either gained or lost, and uses MCMC sampling to provide posterior distribution estimates of current population size ( $N_0$ ), ancestral population size ( $N_1$ ), mutation rate ( $\mu$ ) and time since the population size change ( $T_a$ ). We selected the exponential change model and a generation time ( $g_a$ ) of 3 years as *C. decresii* lives for 2–5 years in the wild (Yewers & D. Stuart-Fox, unpublished data). We set starting values for prior means (on a  $\log_{10}$  scale) for  $N_0$ ,  $N_1$ ,  $\mu$  and  $T_a$  based on our knowledge of the system. Given that current population sizes are expected to be large, we set the prior for  $N_0$  to 4. We also set the  $N_1$  prior to 4 as we have no information on ancestral population size, and populations may have undergone either expansion or contraction. A starting value of  $-3.5$  was used for  $\mu$ , which falls within the range of estimated mutation rates for microsatellite loci

(Goldstein & Schlötterer, 1999; Peery *et al.*, 2012). Finally, we set the  $T_a$  prior to 5 as we expect that changes in population size may be associated with Pleistocene glacial–interglacial cycles. All starting values had a variance of 1. For each population, we performed five independent runs for  $2 \times 10^9$  iterations, thinned at each  $10^5$  interval, with the first 10% of iterations for each run discarded as burn-in. Run convergence was assessed using potential scale reduction factor statistics (values  $< 1.2$ ) calculated in the R CODA package (Brooks & Gelman, 1998; Plummer *et al.*, 2006), and runs were combined using Tracer ver. 1.5 (Rambaut & Drummond, 2009).

### Gene flow between regions

Using the coalescent-based Bayesian framework in Migrate-N ver. 3.6 (Beerli & Felsenstein, 2001; Beerli, 2006, 2009) and microsatellite data, we investigated gene flow between the proximal Olary Ranges and Mt Lofty Ranges geographic regions. Migrate-N estimates mutation-scaled effective population size ( $\theta = 4N_e\mu$ , where  $N_e$  is effective population size and  $\mu$  is mutation rate) and mutation-scaled migration rate from population  $i$  to population  $j$  ( $M_{ij} = m/\mu$ , where  $m$  is the immigration rate per generation), and based on these parameters, the effective number of migrants per generation can be calculated as  $N_e m = \theta_j M_{ij}/4$ . Migrate-N can also be used to compare alternative hypotheses of gene flow between populations based on the marginal likelihood of different models. We evaluated two migration models: (i) full migration to and from the two regions; and (ii) no migration between the two regions.

Preliminary runs based on the more complex model (full migration) were used to determine optimal parameter settings and run lengths. For the final analyses, the Brownian-motion model was employed with four static chains of temperatures 1, 1.5, 3 and  $10^6$ , and swapping among chains allowed every 10 steps. For each model, we performed two independent runs of  $10^6$  recorded steps sampled every  $10^2$  steps, with a burn-in length of  $10^5$  steps. Parameter starting values were based on  $F_{ST}$  estimates, and relative mutation rates of the ten loci were calculated from the data. Given that population sizes are expected to be large and gene flow is expected to be low, we used a uniform prior for  $\theta$  between 0 and 200 ( $\Delta = 20$ ) and a uniform prior for  $M$  between 0 and 50 ( $\Delta = 5$ ). To assess convergence between the two runs, parameter values were compared to ensure overlap of 95% confidence intervals, and ESS values were  $> 8 \times 10^4$  for all parameters. To compare the two migration models, we used the Bezier corrected marginal likelihood to calculate log Bayes Factors and model probabilities (Beerli & Palczewski, 2010). Log Bayes Factor differences of  $> 10$  units provide very strong support for one model over another (Kass & Raftery, 1995).

## Results

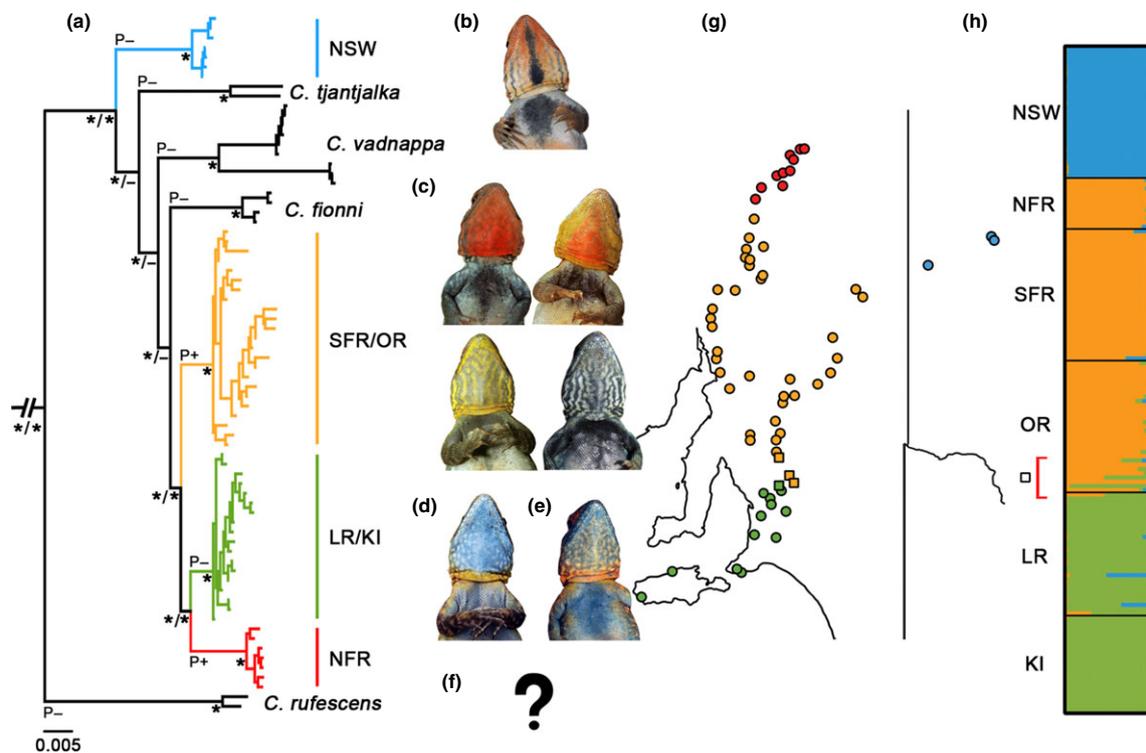
### Colour variation among populations

All seven populations in the Flinders Ranges and the population from the Olary Ranges were polymorphic with four discrete male throat colour morphs, orange, orange and yellow, yellow and grey (Teasdale *et al.*, 2013; Fig. 2c), and exhibited considerable geographic variation in relative morph frequencies. The average frequencies and range of frequencies for each morph across the eight polymorphic populations were as follows: orange: 20% (10–40), orange and yellow: 32.6% (9.5–60), yellow: 31.4% (13.3–61.9) and grey 15.9% (4–36.4). Examination of museum specimens indicated the same four morphs in the Northern Flinders Ranges. Conversely, a single morph existed in each of the remaining populations studied, with the proportion of colours and the degree of patterning showing continuous variation (Fig. S1). In NSW, males had cream coloured throats with varying degrees of orange around

the mouth and a distinct black central stripe (Fig. 2b). In the Mt Lofty Ranges (Fig. 2d) and on Kangaroo Island (Fig. 2e), males had bright blue throats with an ultraviolet reflectance peak and yellow to orange coloration along the gular fold. These two populations could be differentiated, however, with males from the latter having yellow patches dispersed throughout the blue throat coloration (Fig. 2e; Fig. S1). Although variation in the degree of yellow or orange was evident within these populations, this reflected continuous variation within a single morph type rather than discrete morphs like those present in the Flinders and Olary Ranges populations.

### Phylogenetic and phylogeographic structure

Regional colour variation corresponded to phylogeographic structure within *C. decresii*. The combined mtDNA and nuDNA phylogenetic analysis recovered four geographically coherent, reciprocally monophyletic lineages, corresponding to the Northern Flinders Ranges



**Fig. 2** (a) Combined mtDNA and nuDNA sequence data tree, rooted with *Ctenophorus pictus* (not shown), with branch colours representing *Ctenophorus decresii* lineages. Asterisks indicate posterior probabilities > 0.95 generated from MrBayes and \*BEAST analyses, respectively. Single asterisks indicate posterior probabilities > 0.95 from MrBayes only. P+ and P– indicate polymorphic and monomorphic lineages respectively. (b) New South Wales (NSW) male colour morph. (c) Southern Flinders Ranges and Olary Ranges (SFR/OR) male colour morphs; orange, orange and yellow, yellow and grey. (d) Mt Lofty Ranges (LR) male colour morph. (e) Kangaroo Island (KI) male colour morph. (f) Northern Flinders Ranges (NFR) colour variation is unknown; however, museum specimens indicate the same colour polymorphism observed in SFR/OR. (g) Map showing geographic clustering of genetic lineages (coloured), with squares indicating locations of genotypic admixture. (h) STRUCTURE analysis of microsatellite data with individual assignment probabilities for  $K = 3$ . Individuals are arranged in latitudinal order.

(NFR), Southern Flinders Ranges and Olary Ranges (SFR/OR), Mt Lofty Ranges and Kangaroo Island (LR/KI), and western New South Wales (NSW) geographic regions (Fig. 2a,f), which were separated by net Tamura-Nei sequence divergences of 3.7–9.6% in mtDNA (Table S4). Although individual nuclear genealogies exhibited less geographic structuring and phylogenetic resolution, indicative of incomplete lineage sorting and relatively low substitution rates, both the combined nuclear gene tree and mtDNA tree supported polyphyly of *C. decresii* with the NSW lineage positioned more basally in the phylogeny (Fig. S2). This support for polyphyly was not upheld in the species trees reconstructed using phased nuDNA haplotypes in \*BEAST (Fig. 2a; Fig. S3). Nevertheless, there was pronounced genetic divergence (7.1–9.6% net sequence divergence in mtDNA, Table S4) between NSW and the three other *C. decresii* lineages across all data sets, comparable to interspecific net sequence divergence (6.1–9.3%) within the species complex. The geographic distances between adjacent lineages were small, with only 21 km separating the NFR and SFR/OR lineages and 19 km separating the SFR/OR and LR/KI lineages but larger between the SFR/OR and NSW lineage (100 km; Fig. 2f).

Analysis of molecular variance (AMOVA) showed strong genetic differentiation among regions using both sequence (mtDNA and nuDNA) and microsatellite data (all  $P < 0.0001$ ; Table 1). The STRUCTURE analysis based on microsatellite data consistently recovered three lineages ( $K = 3$ ) corresponding to the NSW, LR/KI, and combined NFR and SFR/OR regions (Fig. 2g). These results were largely concordant with the phylogenetic analysis with all individuals clustering into corresponding clades. The exception being that the microsatellite data did not differentiate the NFR from the SFR/OR regions (Fig. 2a,g). The STRUCTURE analysis based on nuDNA sequence haplotypes, however, recovered two lineages ( $K = 2$ ) and showed strong differentiation in individual assignment probabilities between the NFR and SFR/OR regions (Fig. S4). This was consistent with results arising from the phylogenetic analyses. For the microsatellite data set, ENA correction for the presence of null alleles resulted in only a small reduction in global  $F_{ST}$  (Table 1), suggesting that null alleles had a minor impact.

**Table 1** Comparison of overall analysis of molecular variance (AMOVA) results for mtDNA ( $\Phi_{ST}$ ), nuDNA and microsatellite data sets. Identified regions are Northern Flinders Ranges (NFR), Southern Flinders Ranges (SFR), Olary Ranges (OR), Mt Lofty Ranges (LR) and Kangaroo Island (KI).

Population structure	Data type	$F_{ST}$	$F_{ST}^{[ENA]}$	$P$
(NFR) (SFR) (OR) (LR) (KI)	mtDNA	0.915		< 0.0001
	nuDNA	0.112		< 0.0001
	Microsatellite	0.087	0.077	< 0.0001

## Genetic diversity and demographic history

Genetic diversity did not differ significantly between the NFR, SFR/OR and LR/KI lineages for allelic richness ( $A$ :  $F_{2,21} = 0.95$ ,  $P = 0.40$ ) or expected heterozygosity ( $H_E$ :  $F_{2,21} = 0.24$ ,  $P = 0.79$ ). The lineages did differ in observed heterozygosity ( $H_O$ :  $F_{2,21} = 4.25$ ,  $P = 0.028$ ), with the LR/KI lineage having lower observed heterozygosity than the SFR/OR lineage. Genetic diversity also differed among sampled populations for allelic richness ( $A$ :  $F_{4,35} = 4.22$ ,  $P = 0.0068$ ) and observed heterozygosity ( $H_O$ :  $F_{4,37} = 8.15$ ,  $P < 0.0001$ ), but not expected heterozygosity ( $H_E$ :  $F_{4,37} = 1.38$ ,  $P = 0.26$ ). Specifically, the Kangaroo Island population (Snake Lagoon) showed significantly less allelic richness than populations in the Southern Flinders (Aroona and Telowie Gorge) and Olary Ranges (Bimbowrie) and had lower observed heterozygosity than all other populations (Table 2). MSVAR coalescent-based analyses revealed signatures of contraction for all populations, in the order of 97–99% reduction in effective population size, and the timing of this event was consistently between 4000 and 1000 years before present (BP; Fig. 3).

## Gene flow between lineages

The microsatellite-based STRUCTURE analysis indicated potential genotypic introgression between the LR/KI and NFR/SFR/OR lineages, with a small number of admixed individuals present in the most proximal populations of the adjacent Olary Ranges and Mt Lofty Ranges regions (Fig. 2g). Based on the full migration model investigated in Migrate-N, gene flow between the Olary Ranges and Mt Lofty Ranges was symmetrical and extremely low (Table 3). For both directions, the lower 2.5 percentile for  $M$  was equal to 0; thus, we cannot rule out no migration between these regions. However, comparison of migration models revealed that the full migration model had the highest support from the data, with a posterior probability of 1 and a log Bayes Factor difference  $> 10^5$  units relative to the no migration model (where a difference of  $> 10$  units provide very strong support for one model over another; Kass & Raftery, 1995). The estimated value of  $\theta$  was nearly four times larger for the Olary Ranges relative to the Mt Lofty Ranges (Table 3). Therefore, the effective number of immigrants ( $N_{e,m}$ ) moving from the Mt Lofty Ranges to the Olary Ranges was approximately four times larger than the reverse direction (Table 3).

## Discussion

Phylogeographic structure corresponded with geographic colour variation in *C. decresii*. A deeply divergent lineage with a single, unique male throat colour morph was present in the Barrier Range in western

**Table 2** Genetic diversity statistics and demographic analyses of lineages and populations of *Ctenophorus decresii*. Haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) were calculated from mitochondrial sequence data. Observed ( $H_o$ ) and unbiased expected heterozygosity ( $H_E \pm$  standard deviation), and allelic richness ( $A$ ), corrected by the rarefaction procedure to account for uneven sample sizes, were calculated from microsatellite data.

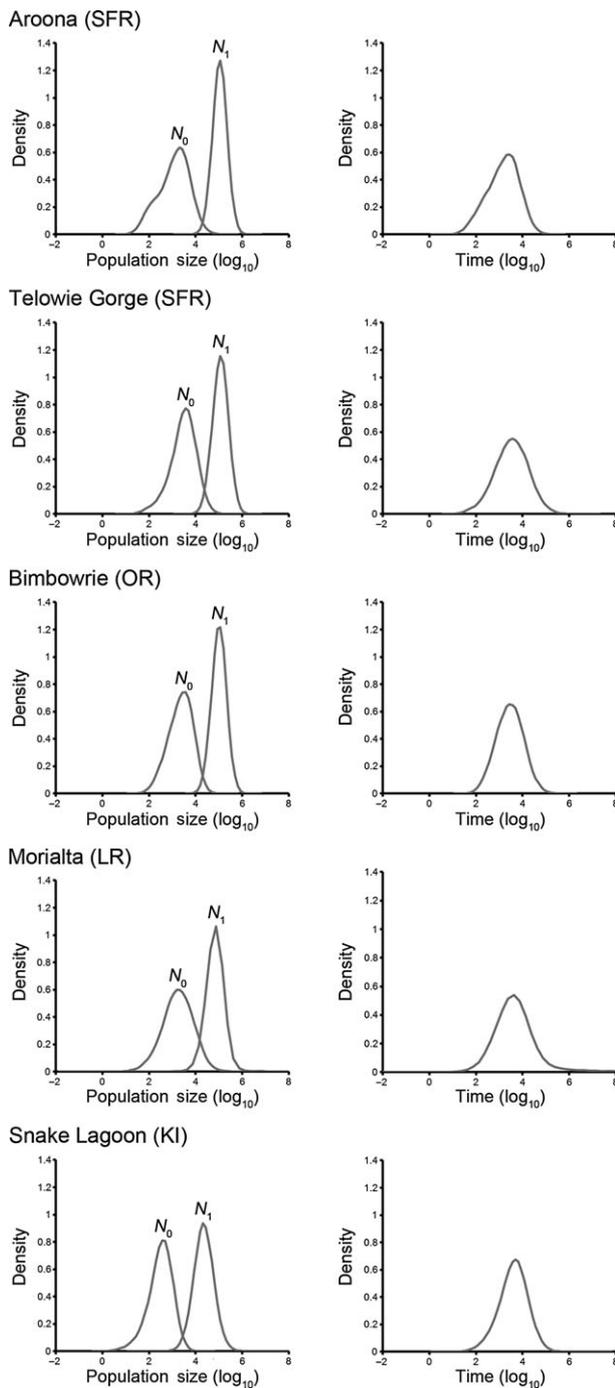
Parameter	Lineage				Population					
	'NFR'	'SFR/OR'	'LR/KI'		Aroona (SFR)	Telowie Gorge (SFR)	Bimbowrie (OR)	Morialta (LR)	Snake Lagoon (KI)	
mitDNA										
Sample size	12	56	25							
$h$	0.727	0.966	0.810							
$\pi$	0.001	0.009	0.003							
Microsatellite										
Sample size	12	142	52	30	30	30	11	22		
$H_o$	0.595 $\pm$ 0.246	0.743 $\pm$ 0.128	0.525 $\pm$ 0.104	0.779 $\pm$ 0.141	0.765 $\pm$ 0.139	0.744 $\pm$ 0.194	0.576 $\pm$ 0.210	0.360 $\pm$ 0.228		
$H_E$	0.843 $\pm$ 0.188	0.875 $\pm$ 0.139	0.855 $\pm$ 0.086	0.855 $\pm$ 0.114	0.836 $\pm$ 0.131	0.827 $\pm$ 0.177	0.741 $\pm$ 0.258	0.690 $\pm$ 0.105		
$A$	11.1	11.2	9.24	6.75	6.69	6.83	5.91	3.62		

New South Wales ('NSW' lineage). Genetic divergence between the 'NSW' and other lineages was comparable to the level of interspecific diversity within the *C. decresii* species complex, and phylogenetic analyses indicate that *C. decresii* is polyphyletic. Morphological analyses have recently confirmed that the 'NSW lineage' represents a separate species (McLean *et al.*, 2013). We therefore do not consider the 'NSW lineage' in further analyses of the phylogeographic and demographic history of *C. decresii*.

There were three major genetic lineages of *C. decresii sensu stricto*, corresponding to the Northern Flinders Ranges ('NFR'), combined Southern Flinders Ranges and Olary Ranges ('SFR/OR'), and combined Mt Lofty Ranges and Kangaroo Island ('LR/KI') geographic regions. Although both mtDNA and nuDNA sequence data clearly separated the 'NFR' and 'SFR/OR' lineages (Fig. 2a; Fig. S2), this division was not supported by microsatellite data (Fig. 2h). Consequently, this discordance is specifically associated with the microsatellite data, and given that the loci used in this study are highly variable, one possible explanation is that homoplasy, due to constraints on allele size and back mutation, has reduced the signature of genetic divergence between these regions (Nauta & Weissing, 1996; Estoup *et al.*, 2002; Selkoe & Toonen, 2006). Although we are unsure of the cause of the discordance between microsatellite and sequence data, our findings reinforce the importance of examining multiple genetic markers and investigating current gene flow in phylogeographic analyses (Godinho *et al.*, 2008; Edwards & Bensch, 2009; DiBattista *et al.*, 2012).

The two northern-most lineages ('NFR' and 'SFR/OR') appear to have the same four male throat colour morphs. By contrast, populations in the 'LR/KI' lineage were monomorphic, with some differentiation in coloration evident between morphs in the two constituent regions (Mt Lofty Ranges and Kangaroo Island). Although we did not specifically survey other species within the complex, none of them are known to be polymorphic (Houston & Hutchinson, 1998). Given the sister relationship between the 'NFR' and 'LR/KI' lineages (Fig. 2), colour polymorphism may have evolved once in the *C. decresii* species complex, in the ancestor of *C. decresii sensu stricto*, and been subsequently lost in the 'LR/KI' lineage. Alternatively, polymorphism may have arisen twice, once in each of the 'NFR' and 'SFR/OR' lineages. Although both scenarios are equally parsimonious, the latter is less likely given the similarity in the nature of the polymorphism and lack of other phenotypic differentiation between Northern and Southern Flinders Ranges populations (McLean *et al.*, 2013). Below, we discuss the evolution and maintenance of colour polymorphism in the light of the biogeographic and demographic history of this species.

Applying a conservative mitochondrial calibration of 2% sequence divergence per million years, the exist-



**Fig. 3** Posterior distributions of current ( $N_0$ ) and ancestral ( $N_1$ ) effective population sizes and time since population size change ( $T_a$ ) represented on a  $\log_{10}$  scale based on combined data from five independent MSVAR simulations for each population.

tence of three major lineages ('NFR', 'SFR/OR' and 'LR/KI'; 3.7–6.4% net sequence divergence in mtDNA) within *C. decresii sensu stricto* is consistent with repeated contraction to, and expansion from, isolated refugia

during Pleistocene glacial–interglacial cycles (Byrne, 2008). Although no obvious geographic barriers exist between the Northern and Southern Flinders Ranges, the climate is more arid in the Northern Flinders Ranges (Schwerdtfeger & Curran, 1996; Brandle, 2001), and the division between the 'NFR' and 'SFR/OR' lineages corresponds to phenotypic divergence observed in rock-dwelling skinks (Chapple *et al.*, 2008) and geckos (Sistrom *et al.*, 2012), and the southerly range limit of the closely related red-barred dragon, *C. vadanappa* (Wilson & Swan, 2010). The Flinders and Mt Lofty Ranges are separated by an expanse of low-lying grassland which we refer to here as the Barossa Valley (Fig. 1). This area is thought to act as a minor geographic barrier for a Bottlebrush shrub (McCallum *et al.*, 2013) and some bird species (Ford, 1987) and was proposed as a current barrier between the northern and southern 'races' of *C. decresii* (Houston, 1974). Nevertheless, our surveys revealed that the species is present throughout the region and that scattered rock aggregates along low-lying ridges currently provide suitable habitat for *C. decresii*, albeit at low population density. We also detected evidence of secondary contact between the 'SFR/OR' and 'LR/KI' lineages with genotypic admixture restricted to peripheral populations in the Olary and Mt Lofty Ranges regions (within 50 km; Fig. 2g,h); however, we did not detect individuals with intermediate colour patterns. Estimates of gene flow between these adjacent regions were extremely low. The STRUCTURE analysis indicated greater genotypic introgression from the Mt Lofty Ranges to the Olary Ranges region. Similarly, the effective number of immigrants ( $N_{em}$ ) moving from south to north (recovered from Migrate-N) was approximately four times larger than the reverse direction. This pattern of asymmetrical gene flow may partly reflect the greater number of individuals sampled from populations directly to the north of the contact site relative to the south and requires further investigation.

Given that throat coloration is likely to be involved in intra- and intersexual selection (Gibbons, 1979; Stuart-Fox & Johnston, 2005) and colour may be involved in species recognition in the *C. decresii* species complex (Houston, 1974), divergence in morph composition between the 'SFR/OR' and 'LR/KI' lineages may act as a prezygotic reproductive barrier. Additionally, the 'SFR/OR' and 'LR/KI' lineages may differ in adaptation to local climatic conditions. The Barossa Valley coincides with a change from relatively more temperate to semiarid conditions, corresponding to range limits of wet-adapted species to the south (e.g. White's skink, *Egernia whitii*; eastern bearded dragon, *Pogona barbata*) and dry-adapted species to the north (e.g. tree skink, *Egernia striolata*; southern spiny-tailed gecko, *Strophurus intermedius*; Wilson & Swan, 2010). Further detailed examination of the contact zone, both in terms of genetic and phenotypic markers, and physiological dif-

**Table 3** Estimates of mutation-scaled effective population size ( $\theta$ ), mutation-scaled migration rate ( $M_{ij}$ ) and effective number of immigrants per generation ( $N_{em} = \theta_j M_{ij}/4$ ) between the Olary Ranges (OR) and Mt Lofty Ranges (LR) regions based on Bayesian analysis in Migrate-N. Migration is from the source region (first column) to the region listed in the first row. Data are modes with the lower 2.5 and upper 97.5 percentiles in parentheses.

Source region	$\theta$	OR		LR	
		M	$N_{em}$	M	$N_{em}$
OR	34.9 (22.9–67.5)	–	–	0.183 (0–1.000)	0.418 (0–2.361)
LR	9.13 (3.73–15.3)	0.183 (0–1.033)	1.595 (0–9.013)	–	–

ferentiation, is needed to resolve the extent and nature of reproductive isolation between the South Australian lineages. Nevertheless, the limited gene flow, in conjunction with the sharp change in phenotype, may suggest potential pre- and/or post-zygotic barriers to gene flow between them. Our study is therefore consistent with other examples showing geographic variation in polymorphism associated with recent genetic divergence (e.g. Messmer *et al.*, 2005; Hull *et al.*, 2010; Iserbyt *et al.*, 2010; Zoppoth *et al.*, 2013). Several polymorphic lizard taxa, for instance, include subspecies which differ in morph composition (Chapple *et al.*, 2008; Corl *et al.*, 2010; Glor & Laport, 2012) and show reduced gene flow between populations that differ in the presence and frequency of morphs (Corl *et al.*, 2012; Bastiaans, 2013).

Demographic analysis of multilocus data revealed a major population decline, across all populations, centred on approximately 3000 BP, corresponding to the late Holocene. This was a period of increased climatic variability and aridity (Reeves *et al.*, 2013), characterized by increased dust deposition and reduced precipitation compared with relatively wetter conditions in the early to mid Holocene (Singh & Luly, 1991; McCarthy *et al.*, 1996; Marx *et al.*, 2009). During this time, populations may have contracted to elevated rocky ranges, which maintain more mesic conditions during arid periods (Byrne, 2008). We are cautious in our interpretation of these demographic results as MSVAR assumes a strict stepwise mutation model, and loci with a high proportion of multistep mutations can produce false signatures of population contraction (Girod *et al.*, 2011). However, MSVAR is robust to moderate departures from this assumption (Girod *et al.*, 2011), and given the observed allele distribution and frequencies of our loci, it is unlikely that our data departs strongly from the SMM.

## Conclusions

Theoretical and empirical evidence suggests that polymorphism can be associated with rapid diversification, giving rise to monomorphic daughter species (Gray & McKinnon, 2007; Forsman *et al.*, 2008; Corl *et al.*, 2010; Hugall & Stuart-Fox, 2012). However, these predictions are difficult to test as polymorphism is gener-

ally rare and phylogenetically scattered (Hugall & Stuart-Fox, 2012). Our comprehensive surveys revealed that within the *C. decresii* species complex, the 'NFR' and 'SFR/OR' lineages of *C. decresii sensu stricto* are the sole polymorphic clades and all constituent populations appear to share the same morph types. Consequently, two possible scenarios exist for the generation/loss of polymorphism in this species. Firstly, polymorphism may have evolved in *C. decresii sensu stricto* and been subsequently lost in the 'LR/KI' lineage, facilitating the evolution and fixation of a new morph type (blue throat). This may be due to changes in the strength and nature of selection associated with the loss of one or more morphs, as selective pressures can vary depending on the number and frequency of morphs present in a population (Sinervo *et al.*, 2000; Sinervo & Clobert, 2003; Svensson *et al.*, 2005). Given that we detected major declines in population size in 'LR/KI' populations, polymorphism may have been lost by chance during population contraction. However, we found no evidence of reduced genetic diversity in the 'LR/KI' lineage (Table 2), which may be associated with polymorphism loss during historical bottlenecks. Furthermore, we detected population declines, of equal severity, in 'SFR/OR' populations (Fig. 3). Alternatively, polymorphism may be a derived state in both the 'NFR' and 'SFR/OR' lineages, maintained by frequency-dependent or balancing selection (e.g. Sinervo & Lively, 1996; Svensson *et al.*, 2005; Takahashi *et al.*, 2010). Both scenarios are equally parsimonious given that they both involve two morph change events; however, independent evolution of similar throat colour polymorphism in the phenotypically undifferentiated 'NFR' and 'SFR/OR' lineages seems less likely. Finally, we found evidence of secondary contact between the 'SFR/OR' and 'LR/KI' lineages with limited genetic admixture and no individuals with intermediate coloration, suggesting potential incipient speciation between populations differing in morph composition. Further research characterizing clines in phenotypic and genetic markers across the contact zone, and on the processes maintaining polymorphism and affecting morph frequencies within the Flinders and Olary Ranges populations of *C. decresii sensu stricto*, will further increase our understanding of the role of polymorphism in speciation.

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## Supporting information

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

**Figure S1** Principle component analyses investigating male throat colour variation in (A) Mt Lofty Ranges (LR; black) and Kangaroo Island (KI; grey), and (B) New South Wales populations of *Ctenophorus decresii*.

**Figure S2** Individual gene trees produced in MrBayes with branches coloured according to lineage, rooted with *Ctenophorus pictus* (not shown).

**Figure S3** Species trees produced in \*BEAST with branches coloured according to lineage, rooted with *Ctenophorus pictus* (not shown).

**Figure S4** STRUCTURE analysis based on phased nuDNA sequence haplotypes ( $K = 2$ ).

**Table S1** List of *Ctenophorus decresii* and out-group samples used for phylogeographic analyses including museum numbers, localities and loci sequenced/genotyped. Samples without museum IDs were collected during fieldwork.

**Table S2** Description of 6 microsatellite loci isolated from *Ctenophorus decresii* screened across 230 individuals with information including primer fluorescent labels (VIC, NED, 6FAM, PET dyes), the number of alleles identified ( $N_A$ ), and the polymerase chain reaction protocol including annealing temperature and Taq DNA polymerase used (PCR).

**Table S3** Characteristics of microsatellite loci when screened across six populations of *Ctenophorus decresii* representative of five main regions.

**Table S4** Net sequence divergence ( $d_A$ , Tamura-Nei) between the northern Flinders Ranges (NFR), southern Flinders Ranges (SFR), Olary Ranges (OR), Mt Lofty Ranges (LR), Kangaroo Island (KI), and New South Wales (NSW) regions for mtDNA (upper diagonal values) and concatenated nuDNA (lower diagonal values).

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