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Effects of vicariant barriers, habitat stability, population isolation and environmental features on species divergence in the south-western Australian coastal reptile community

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Abstract

Identifying explicit hypotheses regarding the factors determining genetic structuring within species can be difficult, especially in species distributed in historically dynamic regions. To contend with these challenges, we use a framework that combines species distribution models, environmental data and multi-locus genetic data to generate and explore phylogeographic hypotheses for reptile species occupying the coastal sand-dune and sand-plain habitats of the south-western Australian biodiversity hotspot, a community which has both a high diversity of endemics and has varied dramatically in spatial extent over time. We use hierarchical AMOVA, summary statistic and distance-based analyses to explicitly test specific phylogeographic hypotheses. Namely, we test if biogeographic vicariance across barriers, habitat stability, population isolation along a linear habitat or fragmentation across different environments can explain genetic divergence within five co-distributed squamate reptile species. Our results show that patterns of genetic variation reflect complex and species-specific interactions related to the spatial distribution of habitats present currently and during repeated glacial minima, as opposed to being associated with historical factors such as habitat stability between glacial and inter-glacial periods or vicariant barriers. We suggest that the large impact of habitat characteristics over time (i.e. relative levels of habitat connectivity, climatic gradients and spatial heterogeneity of soil types) reflects the ecological restrictions of the sand-dune and sand-plain reptile communities and may explain the lack of concordance across taxa. The study demonstrates the general utility of the approach for assemblage-level, as well as single species, phylogeographic study, including its usefulness for exploring biologically informed hypotheses about what factors have influenced patterns of genetic variation.

Keywords: Australian reptiles, comparative phylogeography, multi-locus, species distribution model, statistical phylogeography

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Introduction

Comparative phylogeographic analyses of diverse communities in highly subdivided yet historically dynamic landscapes represent one of the greatest challenges in evolutionary biology. Understanding the historical and ecological factors underlying diversity patterns within a system in a comparative context (e.g. Avise *et al*. 1987; Schneider *et al*. 1998; Hewitt 2000; Riddle *et al*. 2000; Carnaval 2002; Carstens *et al*. 2005) and the processes structuring communities (e.g. Cavender-Bares *et al*.

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2009) both of which are vital for effective conservation management (Moritz & Faith 1998; Moritz 2002). Species distribution models (SDMs; i.e. ecological niche models) are often used for generating phylogeographic hypotheses (Carstens & Richards 2007; Richards *et al.* 2007) in both single taxon (e.g. Hugall *et al.* 2002; Jakob *et al.* 2007; Galbreath *et al.* 2009; Marske *et al.* 2009) and comparative studies (e.g. Carstens & Richards 2007; Waltari *et al.* 2007; Carnaval *et al.* 2009; Moussalli *et al.* 2009). However, SDM techniques do not necessarily encompass all possible hypotheses in a biological credible set of phylogeographic models. The challenge for phylogeographic inference in dynamic and diverse systems comes from the diverse set of hypotheses that represent plausible explanations for diversification, particularly in a comparative context.

Here we use a combined approach that includes SDMs and traditional summary statistics from multilocus genetic data to test a suite of general hypotheses about the factors contributing to the diversity of the south-western Australian coastal reptile community. The south-western Australian coastal community is restricted to coastal sand-dune and plain habitats, is incredibly diverse in reptile species (Schall & Pianka 1978; Storr & Harold 1978, 1980) and flora (Hopper 1979; Crisp *et al.* 2001; Hopper & Gioia 2004) and nested within one of the world's most critically understudied biodiversity hotspots (Cincotta *et al.* 2000; Myers *et al.* 2000). Consequently, understanding the spatial and temporal patterns of diversification within this community is integral to conservation efforts in the region (Moritz & Faith 1998; Moritz 2002) as coastal habitats are under imminent threat from both expanding human development (Del Marco *et al.* 2004) and predicted sea level rises associated with human-induced climate change (Hughes 2003).

Explanations for the diversity of the south-western Australian coastal community focus on the glacio-eustatic changes in climate and sea level in concert with vicariant barriers across the landscape (Storr & Harold 1978, 1980; Hopper & Gioia 2004; Rabosky *et al.* 2004; Edwards 2007; Melville *et al.* 2008). Specific vicariant barriers identified within the region (Fig. 1) have been associated with divergence amongst assemblages (SW/SA—Hopper & Gioia 2004), closely related sister species (South-west—semi-arid transitional zone (SW/SA)—Hopper & Gioia 2004; northern edge of the Victoria Plateau (VP)—Edwards 2007) and amongst deeply diverged lineages within species (Murchison Gorge (MG)—Edwards 2007). Other studies have simply invoked sea level changes and associated changes in spatial habitat extent as responsible for speciation in both plants (Hopper & Gioia 2004) and reptiles (Storr & Harold 1978, 1980; Melville *et al.* 2008) without any detailed analysis of exactly how changes in spatial distribution may have generated diversity.

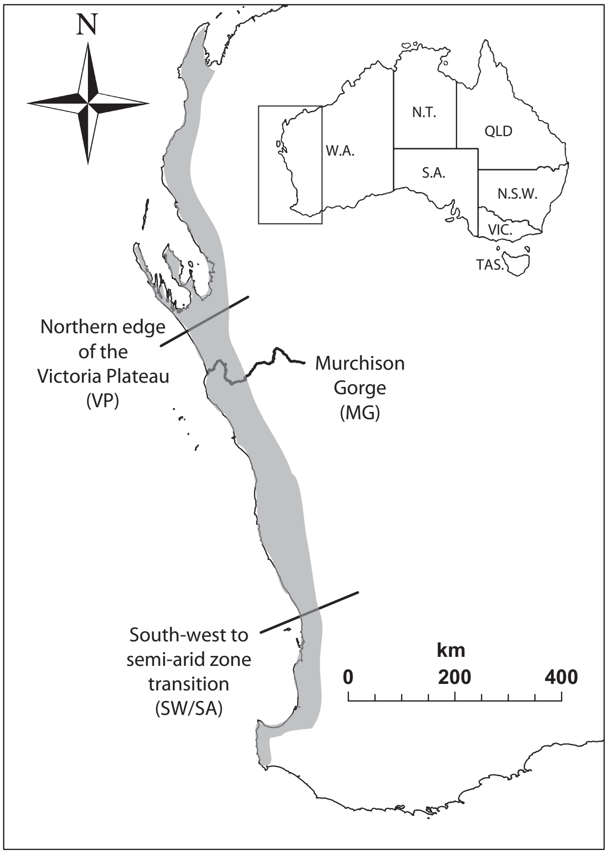

Fig. 1. Map of the south-western Australian sand-plain and sand-dune habitats (shown in grey shading), showing biogeographic features hypothesized to impact phylogeographic structure. Specifically, these include the northern edge of the Victoria Plateau (VP), the Murchison Gorge (MG) and the south-west high rainfall to semi-arid zone transition (SW/SA).

From the early Pleistocene onwards, recession of the Indian Ocean led to the opening up of vast areas of coastal sand habitats along the coastal margin of Western Australia during glacial maxima, while during interglacial periods, species distributions contracted to resemble their current configuration. Processes generating intraspecific diversity within this system could be related to either spatio-temporal expansion/contractions of habitat (Excoffier *et al.* 2009; Knowles & Alvarado-Serrano 2010), vicariant barriers creating long-term barriers to gene flow, areas of habitat stability serving as sources of diversity or isolation by distance along a linearly distributed habitat. Alternatively, given the history and environment of south-western Australia, it is possible that environmental factors may have driven divergence within species. For instance, the current population configuration of species likely represents a protracted state associated with glacial minima (i.e.

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more likely to experience divergence via drift owing to smaller population sizes) in a region with low levels of vegetation community disruption owing to glacial aridity cycles (Dodson 2001) and a mostly stable, pre-Quaternary regolith (with the exception of minor reworking of coastal onshore dunes during the Holocene—Hocking *et al.* 1987). Therefore, it is also possible that intraspecific diversity may be generated by environmental factors limiting dispersal amongst populations (i.e. unsuitable habitats intersecting areas of suitable habitat).

We test the role of vicariant barriers, habitat stability, population isolation and environmental features in contributing to intraspecific divergence within five reptile species sympatrically distributed along the south-western Australian coast (*Diplodactylus ornatus*—ornate stone gecko, *Lucasium alboguttatum*—white-spotted ground gecko, *Morethia lineoocellata*—west coast pale-flecked Morethia, *Lerista lineopunctulata*—line-spotted robust Lerista & *Lerista praepedita*—west coast worm Lerista). Species were specifically selected to represent varying degrees of morphological adaptation consistent with differing levels of ecological specialization and micro-habitat occupation to the sand-plain and dune habitats (detailed below). We discuss our findings in relation to those previously observed within this system and with those observed in comparative studies undertaken in different biomes. We also discuss the utility of this exhaustive framework as a tool for honing model choice for use in model-based phylogeographic inference (Excoffier *et al.* 2009; Knowles 2009; Bertorelle *et al.* 2010).

Materials and methods

Studied taxa

Each of the broadly sympatric focal species differs in life history characteristics and ecological preferences. Amongst the selected species, the two *Lerista* skinks represent a high degree of morphological specialization to the sand-plain and dune habitats; both are obligate fossorial species and lack forelimbs (*L. lineopunctulata*) or both forelimbs and hindlimbs (*L. praepedita*). This morphological specialization is likely to limit dispersal between habitats separated by suboptimal soil profiles, especially for the small worm-like *L. praepedita* (~65 mm) compared to the more robust *L. lineopunctulata* (>100 mm—Cogger 2000; Bush *et al.* 2007; Wilson & Swan 2008). Note that only the northern groups of *L. linepunctulata* are analysed here; the southern groups of *L. lineopunctulata* are the subject of taxonomic revision (Edwards, Doughty & Keogh, unpublished data) and are therefore not included in the phylogeographic analysis of *L. lineopunctulata*. The two diplodactyline gecko species studied have more generalist ecological requirements, as suggested by the extensive distributions of these species and the types of habitats occupied. The two species differ slightly in habitat preferences; both occupying coastal dune and sand-plain habitats, but *Diplodactylus ornatus* is a semi-arboreal species and *Lucasium alboguttatum* is a purely terrestrial species (Cogger 2000; Bush *et al.* 2007; Wilson & Swan 2008). These species also have distributions that extend from the coast to the inland, suggesting they may be better adapted to life in the arid zone and have broader soil preferences compared to the other co-distributed taxa that are limited to the coast. *Morethia lineoocellata* is a eugongyline skink that occupies a range of habitats from coastal dunes and salt flats to sand plains. Its distribution is strictly restricted to a narrow, linear strip along the west coast (Cogger 2000; Bush *et al.* 2007; Wilson & Swan 2008), suggesting this species is only able to persist in the arid zone in the milder conditions afforded by occupying coastal habitats.

Tissues and molecular genetic methods

An average of 64 (±29) individuals was sampled from each species from populations distributed across their respective ranges (for specific sample sizes and sample distribution, see Fig. S1, Supporting information). All tissue samples were obtained from the Western Australian Museum and Australian Biological Tissue Collections (South Australian Museum) (see Table S1, Supporting information). Methods for CTAB genomic DNA extraction, mtDNA (*ND2* gene) amplification and general cycle sequencing protocols followed those outlined in Edwards (2007). PCR protocols for nuclear loci (*PRLR* and *PTPN12*) are outlined in Townsend *et al.* (2008). Primers used for amplification and sequencing of gene products are listed in Table S2 (Supporting information). Sequence data were edited using Sequencher 4.8 (Gene Codes Corporation), aligned using the MUSCLE algorithm (Edgar 2004) in EBIOX (http://www.ebioinformatics.org) and checked by eye. Allelic resolution of nuclear haplotypes was undertaken in DnaSP v5.10 (Librado & Rozas 2009) using PHASE v2.1.1 (Scheet & Stephens 2006). We tested for recombination in nuclear loci using the program SITES (Hey & Wakeley 1997) and inferred recombination only if observed data showed higher levels of recombination when compared to simulated data without recombination (using SEQ-GEN v1.3.3—Rambaut & Grassly 1997) under the model of molecular evolution inferred using JMODELTEST v0.1.1 (Posada 2008) for each data set. Recombination was detected only in the *PRLR* locus in three of the five target species (*Luc. alboguttatum*, *L. lineopunctulata* and *M. lineoocellata*)—see below as to how this was treated

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in each analysis. Distinct haplotype sequences have been logged in GenBank (Table S1, Supporting information).

Environmental niche modelling methods

Climatic data used in SDMs included 19 current climate layers, a categorical soil layer from the Australian Soil Information System (http://www.asris.csiro.au/) and a Global 90 m Digital Elevation Model compiled from data available from Diva-GIS (http://srtm.csi.cgiar.org/). Historical last glacial maximum (LGM) projections were run without the soil layer; soil information is not available for the LGM at lower sea levels. Climate layers were derived from the WorldClim global climate database (available from: http://www.worldclim.org) for current conditions (1950–2000) and the Community Climate System Model for the last glacial maximum (LGM; CCSM v3, Collins *et al.* 2006) and statistically downscaled (Hijmans *et al.* 2005) with data provided by the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2) at a spatial resolution of 10 arc-minutes. The CCSM model was chosen for the LGM climate layers as a global fully coupled model better simulates the El Niño-Southern Oscillation climatic pattern (Bush 2007) that dominates the climate patterns of Australia.

Occurrence data were collated for each of the five focal species from OZCAM (http://www.ozcam.org.au). All data were examined for geo-referencing and misidentification errors: suspect records were excluded to avoid errors in projected distributions (Lozier *et al.* 2009). Distributional records for a total of 176 *M. lineoocellata*, 85 *Luc. alboguttatum*, 204 *L. praepedita*, 139 *L. lineopunctulata* and 81 *D. ornatus* locations were used for the distributional modelling (see Fig. S2, Supporting information for spatial distribution of records used to generate SDMs). Species distributions were estimated using MAXENT v3.3.3a (Phillips *et al.* 2006) based on 10 cross-validation steps using the 'auto features' option with 1000 maximum iterations and a regularization multiplier 1.0 with a 25:75 test/training data ratio. Effectiveness of the model was evaluated using the AUC statistic and area under the receiver operator curve (ROC) characteristics (Peterson *et al.* 2008). The median predicted distributions and habitat suitability scores across the 10 replicate sample distributions were calculated for each species and time period (as detailed below).

Testing the effects of putative biogeographic barriers and habitat connectivity

Analyses of molecular variances (AMOVAS) were used to test the effects of three putative biogeographic barriers in the region (see Fig. 1) and the effects of habitat connectivity on patterns of population differentiation in each species. Three traditionally recognized biogeographic barriers were examined: MG, VP and SW/SA. The effects of each barrier were tested separately (e.g. grouping all populations south vs. north of the MG and testing for significant genetic differentiation between the two groups). Different paired combinations of the barriers, as well as all three barriers acting in concert, were also tested with AMOVAS. Only *M. lineoocellata* and *L. praepedita* were used in tests of the effects of the SW/SA given the limited distributions and/or samples for the other taxa (see Table S1, Supporting information).

To examine the effects of the degree of habitat connectivity of patterns of population differentiation, projections from current species-specific SDMs were used to define populations. Specifically, a population was defined by a region of continuous and highly suitable habitat, such that a population was encompassed by an area with suitability scores >70%, an area >25 km^2, and was separated from other areas of suitable habitat by >25 km (see Knowles & Alvarado-Serrano 2010 for methodological rationale and Fig. S1, Supporting information for distribution of suitable habitat and sample sizes per population). Tests of significant genetic differentiation amongst populations classified according to these criteria provide a means for evaluating the effects of habitat connectivity on species divergence (i.e. tests whether individuals within a region of connected suitable habitat are less distantly related to each other than to individuals from a region separated by areas of unsuitable habitat). If habitat connectivity (present both currently and during repeated inter-glacial periods) is important, AMOVAS should show a large proportion of genetic variation explained by the SDM populations as defined. Although the exact suitability score and size of area for delimiting populations based on habitat connectivity is admittedly arbitrary and less than ideal, for the purposes of the current study only populations in core regions of habitat that could potentially house stable populations were of interest. Moreover, tests of more refined population delimitations (i.e. larger numbers of smaller geographic regions with high habitat suitability) would not be possible with the current sampling.

For all AMOVA analyses, single-phased nuclear haplotypes for each individual were chosen at random and combined with mtDNA sequences prior to analysis. Nonrecombining sections of the *PRLR* locus were treated as independent loci for those species within which recombination was detected (*Luc. alboguttatum*, *L. lineopunctulata* and *M. lineoocellata*). All AMOVAS were conducted in Arlequin v3.5.1.2 (Excoffier & Lischer 2010)

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and permuted 1000 times each to test the significance of fixation indices (ϕ_{ST}'s). Analyses were calculated considering all three loci using the 'locus-by-locus' option, which produces a weighted average for the fixation indices across loci.

Testing the effects of habitat stability

Areas of habitat stability were defined as areas of suitable habitat that were present in both current and LGM SDMs based on contemporary and LGM climatic variables. The limits of current and LGM SDMs were calculated using the maximal test sensitivity-specificity (MSS) threshold as an ecologically relevant threshold of species distribution (Liu *et al.* 2005—see Table S4, Supporting information for values used). Current and LGM-predicted species distributions were compared in ARCGIS 9.3. Areas of overlap between current and LGM-predicted species distributions were used to classify populations as 'stable', whereas areas of the current species distribution not predicted as habitable at the LGM were used to classify populations as 'unstable'. The effect of habitat stability on patterns of genetic differentiation was then tested using hierarchical AMOVAs, with populations grouped according to their stability status (i.e. populations classified as either stable or unstable were grouped together for hierarchical AMOVAs). If habitat stability has contributed to population differentiation, a group effect will be detected, in addition to any genetic variance attributed to amongst population and within populations in the AMOVAs.

Testing for relationships between genetic distance and ecological and geographic factors

Tests for significant relationships between genetic distance, geographic distance and various ecological factors were conducted using distance-based redundancy analyses (dbRDA) with the capscale function of the R package VEGAN (Oksanen *et al.* 2010). The amount of variance in genetic distances explained by environmental variables and controlling for the effects of geographic distance were undertaken using dbRDA conditional analyses (i.e. the relationship between each factor and genetic distance was tested with geographic distance as a covariate). Significance was assessed using 9999 permutations with the ANOVA.CCA function in the VEGAN package (Oksanen *et al.* 2010). In all analyses, genetic distance was treated as the response matrix, which was tested against a series of predictor variables (i.e. geographic distance, climate and soil variables). Here, we are testing the influence of environmental factors present during repeated inter-glacial periods (as opposed to the spatial fluctuations in the extent of coastal habitat between glacial and interglacial periods—see above) on the partitioning of genetic variation. We use current climate and soil layers as a proxy for conditions likely to have been present during various inter-glacial periods during the Quaternary, and across the relevant temporal period over which intraspecific diversity is likely to have been generated within this system. Despite some discussion in the literature on the appropriate use of analytical techniques that combine environmental data with genetic data (Wang 2010; Bohonak & Vandergast 2011), here we combine multilocus sequence data with environmental data to explicitly test our hypothesis that landscape variables may be responsible for intra-specific divergence. This is possible given the comparatively low levels of climate disruption observed during arid phases in south-western Australia (as evidenced by the relative stability of semi-arid plant communities—Dodson 2001) and the antiquity of regolith surfaces in the region (Hocking *et al.* 1987).

Matrices of individual genetic distances were calculated using a Jukes–Cantor correction (Jukes & Cantor 1969) rather than the more commonly used uncorrected-*p* distance to account for multiple substitutions at a site. Individual pairwise distances were calculated for the *ND2* and *PRLR* data separately in MEGA 4.0 (Tamura *et al.* 2007). Where recombination was detected in the *PRLR* locus (i.e. in *Luc. alboguttatum*, *L. lineopunctulata* and *M. lineoocellata*), nonrecombining sections were treated as independent loci. The *PTPN12* locus was not included in these analyses because of limited sampling and low levels of molecular variation (see Fig. S1 and Table S3, Supporting information). Distances were averaged across phased haplotypes within individuals for *PRLR* data and then distances were standardized across loci (i.e. the genetic distance between individuals was divided by the total mean distance per locus to correct for differences in mutation rate across loci) and the average genetic distance of *ND2* and *PRLR* was used for tests of association with the predictor variables (i.e. geographic distance, climate and soil).

Isolation by distance was tested using geographic distance matrices calculated from individual latitude and longitude data using the earth.dist function of the R package FOSSIL (Vavrek 2010). Distance values were first normalized using logarithmic transformation and then converted to a continuous rectangular data set via principal coordinates analyses using the pcnm function of the VEGAN package (Oksanen *et al.* 2010), given that dbRDA analyses cannot analyse matrix predictor variables (see Legendre & Fortin 2010). Information on environmental data for each individual was extracted from 19 WorldClim data layers and a categorical soil data with a resolution of 1 km. To ensure that the

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analyses were not confounded by differences in the scale of environmental variables, data were standardized by subtracting the variable mean and then dividing by the variable standard deviation for each data point prior to analysis. Given the lack of independence amongst climate variables, the first two PC scores from a principal coordinates analysis of all 19 climate variables (conducted with the dudi.pca function of the R package ADE4; Dray & Dufour 2007) were used to test for a relationship between climate and genetic distance.

Results

A detailed summary of the per locus genetic data collected for each species is outlined in Table S3 (Supporting information) and includes the number per locus of the individuals sequenced, base pairs sequenced, haplotypes and summaries of genetic diversity (i.e. S and π). The *ND2* and *PRLR* loci were generally more variable than the *PTPN12* locus. Gene trees (*mtDNA* and *PRLR*), gene networks (*PTPN12*), sample sizes per population, sample distributions and extent of suitable habitat are shown for each species in Fig. S1 (Supporting information). These gene trees show that there are different spatial patterns of divergence across the landscape amongst the distinct species, and therefore little congruence in phylogeographic patterns.

Effects of putative biogeographic barriers and habitat connectivity

Results of tests on the role of biogeographic barriers (i.e. MG, VP and SW/SA; see Fig. 1) on patterns of genetic differentiation show that the impact of traditional biogeographic barriers, relative to the partitioning of genetic variation amongst populations, differs across taxa (Table 1). For example, most of the genetic variation observed in the *Lerista* species is explained by differentiation amongst populations, with relatively small (albeit significant) amounts of differentiation attributable to the

Table 1 Summary results of AMOVAs used to test biogeographic breaks known along the west coast of Australia

		Species									
		Diplodactylus ornatus		*Lucasium alboguttatum*		*Lerista lineopunctulata*		*Lerista praepedita*		*Morethia lineoocellata*	
Scenario	Source	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ
MG	b/w groups	**70.81**	**0.71*****	10.71	0.11n.s	5.99	0.06***	11.89	0.12***	30.59	0.31***
	w/in group	9.35	0.32***	28.82	0.32***	32.20	0.34***	39.23	0.45***	44.08	0.64***
	b/w pops.	19.84	0.80***	60.47	0.40***	61.82	0.38***	48.88	0.51***	25.32	0.75***
VP	b/w groups	**51.42**	**0.51*****	25.53	0.26***	3.48	0.03 n.s.	16.73	0.17***	25.75	0.26***
	w/in group	26.61	0.55***	10.67	0.14***	32.11	0.33***	38.17	0.46***	47.66	0.64***
	b/w pops.	21.97	0.78***	63.81	0.36***	64.40	0.36***	45.09	0.55***	26. 59	0.73***
SW	b/w groups	N/A	N/A	N/A	N/A	N/A	N/A	2.14	0.02 n.s.	**57.79**	**0.58*****
	w/in group	N/A	N/A	N/A	N/A	N/A	N/A	47.56	0.49***	17.80	0.42***
	b/w pops.	N/A	N/A	N/A	N/A	N/A	N/A	50.30	0.50***	24.41	0.76***
MG + VP	b/w groups	**64.43**	**0.68*****	30.04	0.30***	2.77	0.03 n.s.	18.60	0.19***	27.24	0.27***
	w/in group	10.40	0.33***	11.14	0.16***	34.56	0.36***	35.40	0.43***	47.04	0.65***
	b/w pops.	21.17	0.79***	58.82	0.41***	62.66	0.37***	46.00	0.54***	25.72	0.74***
MG + SW	b/w groups	N/A	N/A	N/A	N/A	N/A	N/A	0.71	0.01 n.s.	**57.56**	**0.58*****
	w/in group	N/A	N/A	N/A	N/A	N/A	N/A	48.49	0.49***	16.94	0.40***
	b/w pops.	N/A	N/A	N/A	N/A	N/A	N/A	50.80	0.49***	25.50	0.75***
VP + SW	b/w groups	N/A	N/A	N/A	N/A	N/A	N/A	−5.44	0 n.s.	**54.97**	**0.55*****
	w/in group	N/A	N/A	N/A	N/A	N/A	N/A	55.98	0.53***	18.31	0.41***
	b/w pops.	N/A	N/A	N/A	N/A	N/A	N/A	49.46	0.51***	26.72	0.73***
MG + SW + VP	b/w groups	N/A	N/A	N/A	N/A	N/A	N/A	−2.07	0 n.s.	**55.39**	**0.55*****
	w/in group	N/A	N/A	N/A	N/A	N/A	N/A	53.25	0.52***	18.81	0.42***
	b/w pops.	N/A	N/A	N/A	N/A	N/A	N/A	48.82	0.51***	25.81	0.74***

The proportion of variation (% Var.) and fixation indices (ϕ) are shown to indicate the level of variation explained by groups of populations grouped on either side of biogeographic barriers (b/w groups), amongst populations within areas defined by biogeographic barriers (w/in group) and between populations regardless of grouping level (b/w pops.). Values bolded indicate biogeographic barriers account for >50% of the genetic variation within a species. Abbreviations: MG, Murchison Gorge; VP, northern border of the Victoria Plateau; SW, transition between wet south-west and the semi-arid zone. n.s, $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

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geographic barriers (Table 1). In contrast, *Diplodactylus ornatus* and *Morethia lineoocellata* both have significant and large amounts of genetic variation explained by the geographic barriers, with relatively little genetic variance explained by populations per se (Table 1).

Habitat connectivity (as characterized by populations delimited by the SDMs; see Materials and methods for details) had a much more consistent effect across taxa compared to vicariant barriers (Table 2). In all species, the populations identified from the SDM partitioning strategies accounted for moderate to large amounts of genetic differentiation (Table 2), suggesting that the lack of habitat connectivity between regions of high habitat suitability plays a large role in partitioning genetic variation. Even though the regional population breaks defined by patterns of habitat connectivity are correlated with some of the traditional biogeographic barriers (see Figs 1 and 2), the association cannot explain the significant impact of habitat connectivity on patterns of genetic differentiation. For example, in both *Lerista* species a much larger effect of habitat connectivity on patterns of genetic variation is observed (Table 2) compared to the effects of any geographic barrier considered alone or in combination (Table 1). Note that statistical measures indicate that predicted distributions used to characterize habitat connectivity for each species (Fig. 2) are highly accurate (Table S4, Supporting information) and the projected distributions closely match published accounts of species distributions (Cogger 2000; Wilson & Swan 2008).

Effects of habitat stability

Although SDMs for current conditions show high levels of accuracy in all statistics reported (Table S4, Supporting information), the predicted distributions of species differ depending on whether soil characteristics are included (Fig. 2 and Fig. S3a, Supporting information respectively). Predicted species distributions for current conditions vs. the LGM (Fig. S3b, Supporting information) show that species differ in the extent to which they would have experienced climate-induced distributional shifts, with some species showing pronounced differences in the relative size of contemporary and past ranges. As a result, the species differ in the degree of habitat stability over time (Fig. 3). For example, the gecko species (*D. ornatus* and *Lucasium alboguttatum*) show dramatic increases in past distributional ranges, with large areas of habitat stability (i.e. areas with high levels of suitability in both the present and past), particularly in the Shark Bay and Carnarvon regions. *L. lineopunctulata* and *L. praepedita* also show moderate shifts in distribution (Fig. S3b, Supporting information) but very little habitat stability, with large proportions of the current species' distributions predicted as unstable and a few small patches predicted as refugial (Fig. 3). *M. lineoocellata* shows more moderate levels of climate-induced distributional shifts (Fig. S3b, Supporting information) with an intermediate geographic extent of habitat stability (Fig. 3), relative to the aforementioned species. This species is predicted to have continuously occupied more extensive areas of habitat in the north, as well as a smaller number of disjunct areas of stable habitat in the south. There is also a disjunction between southern and northern populations during the LGM not observed in the current distributional models.

When populations are grouped according to habitat stability, hierarchical AMOVA analyses indicated that habitat stability was not the primary factor structuring patterns of genetic variation (i.e. little genetic variance is explained by stable vs. unstable population groupings). Instead, most of the genetic variance was associated with the difference amongst populations delimited by breaks in suitable habitat (Table 3). There is also no evidence of elevated genetic diversity (θ_S) of populations from historically stable regions, indicating that habitat stability itself is not associated with larger effective population sizes (Table 3).

Table 2 Results of AMOVAs for the effects of habitat connectivity [as characterized by populations delimited by the species distribution models (SDMs), see Materials and methods for details] on patterns of genetic differentiation, including the proportion of variation (% Var.) and the fixation indices (ϕ) between populations grouped by habitat suitability (b/w pops) and amongst individuals within populations (w/in pops)

		Species									
		Diplodactylus ornatus		*Lucasium alboguttatum*		*Lerista lineopunctulata*		*Lerista praepedita*		*Morethia lineoocellata*	
Scenario	Source	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ	% Var.	ϕ
SDM	b/w pops	74.08	0.74***	31.65	0.31***	34.80	0.35***	48.98	0.49***	71.08	0.71***
	w/in pops	25.92		68.35		65.20		51.02		28.92	

n.s, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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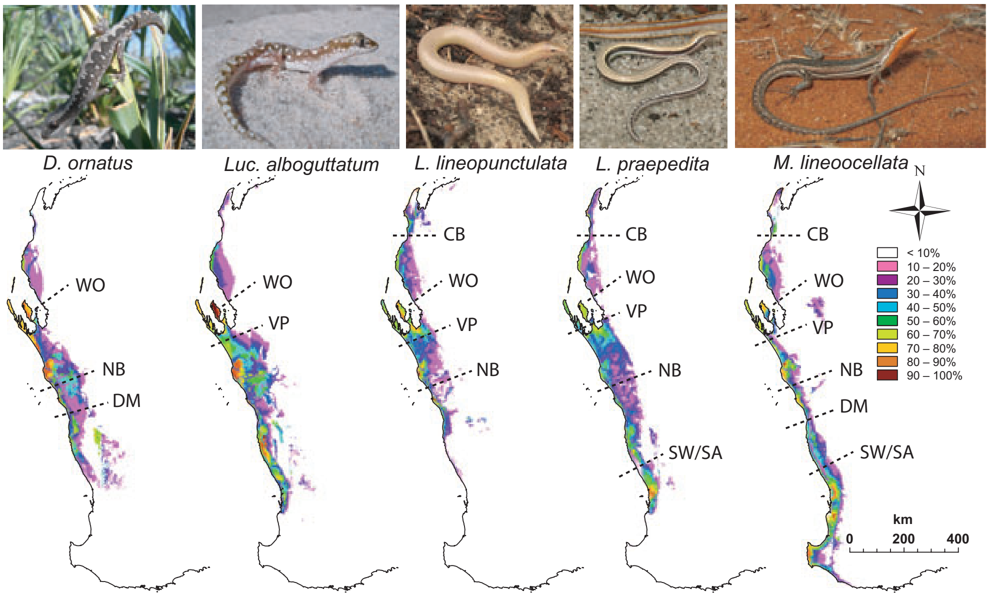

Fig. 2. Predicted current distributions for the five studied taxa; habitat suitability scores per 10 percentile intervals are shown. Regional populations delimited by the species distribution models (SDMs; see text for details) are marked by dashed lines with many distributional breaks in common across species; the number of regional populations recognized from the SDMs varied across species from three to six populations (*Diplodactylus ornatus*—4; *Lucasium alboguttatum*—3; *Lerista lineopunctulata*—5; *Lerista praepedita*—6; *Morethia lineoocellata*—7, of which six were sampled). The labels identify geographic features/localities; specifically, from north to south these include: CB, Coral Bay; WO, Wooramel; VP, Victoria Plateau; NB, Northampton Block; DM, Dongara-Morawah; and SW/SA, South-west—Semi-arid Transitional Zone. Photographs: *Luc. alboguttatum*—D. Edwards; *D. ornatus*—R. Heaton; *L. praepedita*, *L. lineopunctulata* and *M. lineoocellata*—R. Lloyd.

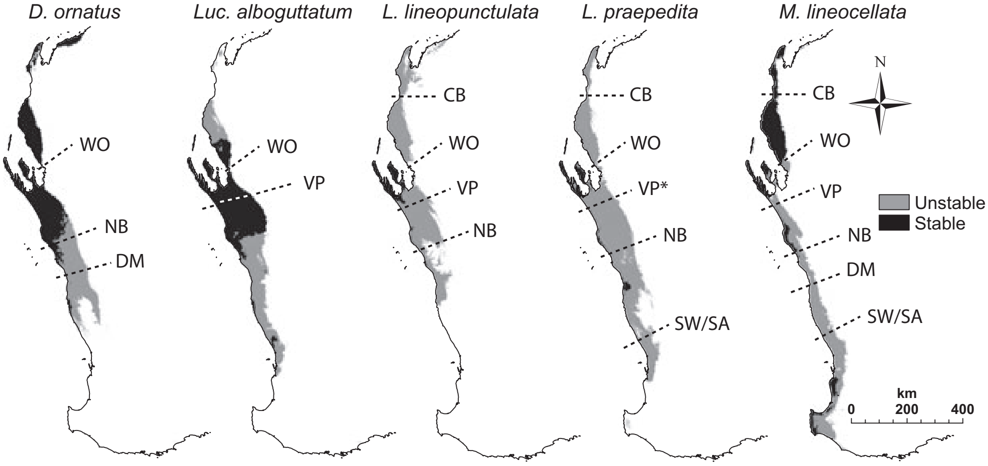

Fig. 3. Habitat stability for five focal reptile taxa of the south-western Australian coastal reptile community. Ecological niche modelling was undertaken in MaxEnt with 19 WorldClim climatic layers and a digital elevation model (corrected for last glacial maximum (LGM) conditions for projections), and the maximal test sensitivity and specificity threshold (Table S4, Supporting information) was applied to both current and LGM models (raw output in Fig. S1, Supporting information). Areas in black indicate overlap between the LGM and current day distributions and represent refugial habitats that are predicted to have been continuously occupied throughout the Pleistocene fluctuations. Areas in grey indicate those areas likely to have fluctuated in and out of habitability for each of the target taxa during inter-glacial and glacial periods, respectively.

Relationship between genetic distance and ecological and geographic factors

All species show a significant relationship between genetic divergence and the individual ecological variables that characterize climatic and soil characteristics (Table 4). Soil characteristics were the most significant predictor variable for all species explaining large amounts of genetic variance (10–47% genetic variance explained) both in marginal and conditional tests using

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Table 3 Summary results of AMOVAS and summary statistics (ϕ_{ST} and θ_S) testing the role of habitat stability

Species	Source	% Var.	ϕ	ϕ_{ST} b/w *U*	ϕ_{ST} b/w *S*	θ_S (*S*/*U*)
Diplodactylus ornatus	b/w groups	25.53	0.26***	0.46	0.77	9.2/22.3
	w/in group	54.46	0.73***			
	b/w pops.	20.01	0.80***			
Lucasium alboguttatum	b/w groups	−13.76	0 n.s.	0.48	0.54	6.3/10.1
	w/in group	47.98	0.42***			
	b/w pops.	65.78	0.34***			
Lerista lineopunctulata	b/w groups	−13.81	0 n.s.	0.36	0.69	13.5/17.5
	w/in group	47.34	0.42***			
	b/w pops.	66.48	0.34***			
Lerista praepedita	b/w groups	13.56	0.14***	0.46	0.69	14.9/37.7
	w/in group	45.95	0.53***			
	b/w pops.	40.48	0.60***			
Morethia lineoocellata	b/w groups	−28.16	0 n.s.	0.65	0.67	20.4/14.9
	w/in group	93.57	0.73***			
	b/w pops.	34.60	0.65***			

Individuals are initially partitioned according to species-specific species distribution model populations and these populations are then split into a stable or unstable populations. Populations are then hierarchically grouped according to classification of stable (*S*) vs. unstable (*U*) populations. The proportion of variation and the fixation indices explained by groups of stable vs. unstable populations (b/w groups), within groups of stable or unstable populations (w/in group) and between populations regardless of stable/unstable classification (b/w pops). Mean θ_π values, mean pairwise ϕ_{ST}'s for pairs of stable and unstable populations and all stable populations are also shown. n.s, $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

geographic distance as a cofactor (Table 4). Climate PC axis 1 was also a significant factor explaining variance in genetic distance for all species (5–28% variance explained—Table 4). Only minor amounts of genetic variance could be explained by climate PC2 in specific taxa (~3% in *L. praepedita* and *Luc. alboguttatum*, respectively—Table 4). Lastly, no signal of isolation by distance was evident in any of the species.

Discussion

This study highlights the potential for considerable variation in the genetic consequences of climate-induced distributional shifts amongst species of the south-western Australian reptile community and the analyses hint at how species-specific ecological preferences have contributed to the observed patterns. Despite this variation, there are general landscape factors (present both currently and during repeated inter-glacial periods) that are influential and broadly applicable in explaining the distribution and maintenance of genetic diversity. Nevertheless, the geological history of the region has had limited influence, and in contrast to tropical herpetofauna (e.g. Carnaval *et al.* 2009), habitat stability is not a predominant factor structuring the assemblage. Instead, ecological factors (including habitat suitability, soil heterogeneity and climatic gradients) are identified as having significant relationships with genetic divergence. More specific findings are discussed below, as is the utility of this methodological framework for the identification of biologically relevant hypotheses for phylogeographic studies with limited a priori information available.

Why do some factors predominate across species?

Our results across multiple species suggest that inhospitable terrain (low levels of habitat suitability in SDMs) owing to unsuitable climatic conditions and soil types, rather than long-term habitat stability, IBD or vicariance explains divergence patterns in this system. The SDM results show many of the habitat breaks indicated in the current study and inferred as barriers in previous studies (Rabosky *et al.* 2004; Edwards 2007; Melville *et al.* 2008) correlate with areas of unsuitable habitat in some but not all species, making vicariance an unlikely explanation for divergence in any one species (Figs 1 and 2). It is possible that breaks in habitat suitability could represent significant transitions in ecological conditions and/or unsuitable divisions between suitable habitat. However, quantitative tests between these competing hypotheses were not possible given the wide gaps between suitable regions, and consequently, a lack of individuals that could be sampled within unsuitable regions (Glor & Warren 2011). The SW/SA transitional zone is a well-known biogeographic transition point (Hopper & Gioia 2004), while other more specific breaks are likely to represent species-specific variation in habitat requirements or ecological similarity for those that are congruent amongst species (Fig. 2).

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Table 4 Tests for the relationship between genetic distance within coastal reptile species and several sets of environmental predictor variables using distance-based redundancy analyses multivariate *F*-statistics, including the proportion of multivariate genetic variation attributed to each variable (% Var)

	Marginal tests				Conditional tests			
Species	Variable	*F*	*P* value	% Var.	Variable	*F*	*P* value	% Var.
Diplodactylus ornatus	Distance	1.72	n.s.	1.15				
	Climate PC1	12.95	***	5.73	Climate PC1	20.17	***	6.91
	Climate PC2	0.22	n.s.	0.16	Climate PC2	0.62	n.s.	0.42
	Soil	5.85	***	10.86	Soil	6.15	***	10.30
Lucasium alboguttatum	Distance	1.91	n.s.	1.78				
	Climate PC1	10.48	***	7.98	Climate PC1	11.31	***	8.21
	Climate PC2	3.26	**	2.94	Climate PC2	3.19	*	2.81
	Soil	5.39	***	23.57	Soil	6.00	***	23.44
Lerista lineopunctulata	Distance	0.91	n.s.	0.77				
	Climate PC1	6.25	***	5.00	Climate PC1	6.35	***	5.07
	Climate PC2	2.09	n.s.	1.75	Climate PC2	2.08	n.s.	1.75
	Soil	3.63	***	31.68	Soil	3.61	***	31.51
Lerista praepedita	Distance	1.35	n.s.	0.74				
	Climate PC1	38.58	***	13.56	Climate PC1	39.34	***	13.58
	Climate PC2	5.85	**	3.02	Climate PC2	6.79	***	3.44
	Soil	4.95	***	22.96	Soil	6.33	***	24.58
Morethia lineoocellata	Distance	0.71	n.s.	0.62				
	Climate PC1	55.64	***	28.10	Climate PC1	56.15	***	28.2
	Climate PC2	1.38	n.s.	1.20	Climate PC2	1.41	n.s.	1.23
	Soil	7.46	***	47.07	Soil	7.41	***	46.77

Marginal tests are indications of the relationship between response variable genetic distance and the predictor variable alone, where conditional tests include geographic distance as a covariate in the analysis. *P*-values: n.s., $P > 0.05$; *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

The impact of species-specific phenomena (Tables 2 and 4, Fig. 2 and Fig. S1, Supporting information) rather than a predominance of shared historical refugia and vicariance explanations contrasts with other recent phylogeographic studies on spatially and temporally dynamic habitats based on coupling SDM projections with genetic data (Hugall *et al.* 2002; Graham *et al.* 2006; Carnaval & Moritz 2008; Carnaval *et al.* 2009; Moussalli *et al.* 2009; Bell *et al.* 2010). Although there are some limitations associated with SDMs (e.g. the lack of a soil layer for LGM SDM modelling, Austin & Van Niel 2011; difficulties in modelling species with shifting distributions, Elith *et al.* 2011; dynamic rather than static refugia, Knowles & Alvarado-Serrano 2010), the fact that soil and climate explain such a large proportion of genetic variation in all species (Table 4) indicates that this result may well be a reliable representation of the factors underlying diversity within this system.

Soil and climate are critical determinants of fitness, survival and dispersal capabilities in reptiles, particularly in species occupying specific habitats. For example, the observed strong relationship between soil and genetic distance (Table 4) is entirely expected in species restricted to sandy habitats through fossorial habit, such as the *Lerista* species. Moreover, substrate choice has been identified as a critical factor in determining phenotypic plasticity in cryptic coloration (Merilaita *et al.* 1999; Stuart-Fox *et al.* 2004; Gray & McKinnon 2007) and thermoregulation (Melville & Schulte 2001) in reptiles. Therefore, these key fitness traits may also be driving dispersal limitation related to soil type. Climate variables are also key features predicting the seasonal activity and reproduction cycles of ectotherms, which biophysical models indicate as critical components predicting spatial distributions (Kearney & Porter 2009) and responses to climatic change (Kearney *et al.* 2009). Therefore, these variables are likely to be controlling factors in determining both the habitat suitability and dispersal ability of a species according to the specific ecological requirements of that taxon, and thus the key drivers of species-specific population differentiation in the south-western Australian coastal reptile community.

Species-specific responses as a generalizable finding to south-western Australian phylogeography?

The biodiversity hotspot of south-western Australia is considered somewhat of an enigma. Relatively subdued

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topographical features of the landscape and the lack of prominent geologic events since the Miocene-Pliocene make it difficult to posit hypotheses about diversification within the region. The entire landscape has experienced dramatic shifts in distributional extent during the Pleistocene glacial—interglacial cycling, and from SDMs some species may well have also undergone spatial shifts in distribution (Fig. S2, Supporting information). Moreover, these changes were especially dramatic along the coast of Western Australia and shaped by a complex interaction of climatic fluctuation and sea level change (Van de Graaff *et al.* 1980; Butcher *et al.* 1984; Hocking *et al.* 1987; Mory *et al.* 2003). Our results suggest that the evolutionary history of south-western Australian coastal reptiles, a seemingly simple linear system, appears to have been fairly species-specific, as opposed to general community-level impacts of past climatic or geologic events. This is contrary to the findings of several previous studies assessing the impacts of geological activity (Hocking *et al.* 1982, 1987) on diversification in the region (Rabosky *et al.* 2004; Edwards 2007; Melville *et al.* 2008). Nevertheless, our findings, in addition to previous studies within the south-western Australian coastal system, failed to find consistent patterns of phylogeographic structure across species (see Fig. S1, Supporting information) suggesting that the notion of species-specific responses may indeed be generalizable to other taxa in the region.

The distribution of the coastal sand-plain and dunal ecosystem (Fig. 1), noted both as a hub of plant (Hopper 1979; Crisp *et al.* 2001; Hopper & Gioia 2004) and reptile diversity (Storr & Harold 1978, 1980), is directly linked to sandy soil types. The ecosystem also encompasses extensive climatic gradients from the wetter south-west high rainfall province to the arid Cape Range regions. Soil and climate have been identified as important constituents of species niches and fitness, and therefore important predictors of species distributions, across taxonomic classes from mammals (Majerus & Mundy 2003; Martínez-Meyer *et al.* 2004) to plants (Austin 2002), not just reptiles. Therefore, we posit that species-specific responses related to the spatial distribution of soil and climate factors that exist within the environment today and have been present during repeated glacial minima are a key factor underlying the generation of population differentiation across the south-western Australian coastal ecosystem.

To explore this hypothesis, further future work in the south-western Australian coastal system should focus on resolving questions about the nature of habitat barriers (e.g. are all habitat barriers representative of significant environmental transitions or simply areas of sustained unsuitable habitat?) in addition to questions on the exact impact of temporal dynamism (e.g. the utility of spatially and ecologically explicit models in predicting species responses to glacio-eustatic fluctuations in sea levels and habitat distributions). More extensive studies at finer spatial scales may also lend support to the hypothesis that climate and soil are key components of dispersal limitation in these species by showing limited contemporary gene flow between areas of low habitat suitability. Our results also have impacts on phylogeographic studies in dynamic systems in general. We have shown that it is possible to combine environmental data with multi-locus sequence data to test phylogeographic hypotheses under the right conditions (i.e. climate layers are an appropriate proxy for the historical period of interest and markers have evolved differences over the same period). Further, despite a tendency in comparative phylogeography to focus on identifying common responses to barriers, when species-specific responses and associations between genetic structure and environmental variables are considered, a clearer picture of the factors underlying diversity becomes more apparent.

Identifying biologically relevant hypotheses in phylogeographic studies

Model choice is a key impediment to the development of biologically realistic hypotheses and deciphering which analytical avenue is most appropriate for the data (Knowles 2009). Full likelihood/Bayesian methodologies (Nielsen & Beaumont 2009; Hey 2010) are of limited use when models are complex, yet both these and approximate methods (Beaumont *et al.* 2010; Bertorelle *et al.* 2010; Csilléry *et al.* 2010) require realistic sets of models to determine which methodology should be used and if either of these methods will be effective in distinguishing between alternative hypotheses. The approach used here provides a means to systematically test for the relevance of biogeographic and ecological factors for inclusion in biologically realistic hypotheses tested using more rigorous techniques. The factors identified in our study could similarly be used to inform the models we consider, expanding on previous studies using SDMs to inform hypotheses (Hugall *et al.* 2002; Carstens & Richards 2007; Jakob *et al.* 2007; Knowles *et al.* 2007; Richards *et al.* 2007; Waltari *et al.* 2007; Carnaval *et al.* 2009; Galbreath *et al.* 2009; Marske *et al.* 2009; Moussalli *et al.* 2009). As a means to generate suites of models that might be considered, such hypotheses are easily applicable to assemblages-level or single species phylogeographic studies, particularly in complex systems without clear a priori hypotheses, and provide a powerful set of tools for narrowing models down to a biologically realistic set and for informing priors on cross-taxon congruence.

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Data accessibility

Sample Information—uploaded as online supporting information.

DNA Sequences: JQ517723–JQ518192.

Distribution records & environmental data used in SDM modelling—Dryad doi:10.5061/dryad.8p7435hq.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 List of genetic samples used in study including genes sampled, Museum from which tissues were subsampled, corresponding museum number identifier, sampling location and associated genbank number.

Table S2 Primer sequences and references.

Table S3 Summary of the multi-locus genetic data for each of the five focal taxa (*D. ornatus*, *Luc. alboguttatum*, *L. lineopunctulata*, *L. praepedita* and *M. lineoocellata*); for each locus the number of base pairs sequenced per locus, individuals sequenced, haplotypes, segregating sites (*S*), and the per site nucleotide diversity (π) are shown.

Table S4 Summary of accuracy and threshold statistics from MaxEnt modeling of species distributions for each of the five coastal reptiles.

Fig. S1 Presented within are the gene trees/networks, sample distributions, SDM distribution of areas with >70% habitat suitability (shown in gray shading on distribution map), population sizes for each of the SDM defined populations and the location of population breaks inferred from SDMs for each species (A–E—*D. ornatus*, *Luc. alboguttatum*, *L. lineopunctulata*, *L. praepedita* and *M. lineoocellata* respectively). Bayesian analyses were undertaken using MrBayes v3.2 (Ronquist *et al.* 2011) and running for 10 million generations with a 25% burnin.

Fig. S2 Spatial distribution of vetted records taken from OZCAM (http://www.ozcam.gov.au) for each species and used in species distribution model generation.

Fig. S3 Current (a) and LGM (b) species distribution models for each species used in the calculation of habitat stability maps (Fig. 3).

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