



The genetic legacy of aridification: Climate cycling fostered lizard diversification in Australian montane refugia and left low-lying deserts genetically depauperate

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ABSTRACT

It is a widely held assumption that populations historically restricted to mountain refugia tend to exhibit high levels of genetic diversity and deep coalescent histories, whereas populations distributed in surrounding low-lying regions tend to be genetically depauperate following recent expansion from refugia. These predicted genetic patterns are based largely on our understanding of glaciation history in Northern Hemisphere systems, yet remain poorly tested in analogous Southern Hemisphere arid systems because few examples in the literature allow the comparison of widespread taxa distributed across mountain and desert biomes. We demonstrate with multiple datasets from Australian geckos that topographically complex mountain regions harbor high nucleotide diversity, up to 18 times higher than that of the surrounding desert lowlands. We further demonstrate that taxa in topographically complex areas have older coalescent histories than those in the geologically younger deserts, and that both ancient and more recent aridification events have contributed to these patterns. Our results show that, despite differences in the details of climate and landscape changes that occurred in the Northern and Southern hemispheres (ice-sheets versus aridification), similar patterns emerge that illustrate the profound influence of the Pleistocene on contemporary genetic structure.

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1. Introduction

Considerable research attention has focused on the evolution of Northern Hemisphere taxa in response to glacial cycles (Schaffer et al., 2010), driven in part by the classic review by Hewitt (2000). In particular, an increasing number of molecular studies have highlighted the importance of Pleistocene refugia in generating and sustaining genetic diversity in boreal and temperate systems (Stewart et al., 2010), as species repeatedly contracted their distributions in response to the periodic extension of ice sheets (Hewitt, 1999), or the contraction and dissection of tropical and temperate forests (Moritz et al., 2000). Despite this recent work, we are only just beginning to scratch the surface in our understanding of climate-induced biotic diversification in arid systems (Beheregaray, 2008). Few studies have directly addressed the impact of aridification on evolutionary history (Bowie et al., 2006), or the role of refugia in arid biomes recently inundated by sand deserts (Toon et al., 2007).

Arid conditions are thought to have been prevalent during the Mid-Miocene associated with global Cenozoic cooling (Flower and Kennett, 1994), so processes underpinning modern

evolutionary patterns of arid-zone taxa are likely to have a deep history (Melville et al., 2009). However, the initiation of glacial cycles in the Plio/Pleistocene caused immense global change (Clark et al., 2006), and the spread of continental ice-sheets across the Northern Hemisphere during glacial cycles was concomitant with cool, arid periods over mid-latitude continental regions. In addition, the transition from high-frequency, low-amplitude glaciations (every 40,000 years [ka]) that characterized the late Pliocene/early Pleistocene, to the low-frequency, high-amplitude glaciations (every 100 ka) that became established in the middle Pleistocene (Huybers, 2007) led to increasingly severe aridification and the development of sand deserts on immense scales (Hartley and Chong, 2002; Fujioka et al., 2009; McLaren and Wallace, 2010). Such dynamic landscape changes over large continental areas would have had a profound effect on the evolution of biota that inhabited these regions, with refugia undoubtedly playing a major role.

Refugia typically occur in regions with heterogeneous topography and serve as centers of persistence for both warm and cold-adapted species by retaining relative climatic stability during periods of hostile climate (Hewitt, 1999). While the refugial concept implicitly involves a reduction in population size (bottleneck) (Bennett and Provan, 2008), diversity has been shown to accumulate over time through the repeated movement of populations both in and out of the refugium, as well as over altitudinal gradients.

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The resulting pattern is one of increased genetic diversity in topographically complex regions, with taxa displaying deep coalescent histories (Hewitt, 1999). Refugia also serve as sources from which adjacent regions were recolonized following amelioration of climatic conditions during interglacial periods. Examination of genetic signatures of Northern Hemisphere post-glacial colonizers has revealed insights into routes of colonization and the mode and tempo of dispersal (Taberlet et al., 1998). The most recent expansion episode followed the retreat of ice-sheets and permafrost after the Last Glacial Maximum <20,000 years ago (Clark et al., 2009). Coupled with this recent history, where post-glacial expansion occurs rapidly, leading-edge expansion can result in large geographic areas that contain low genetic diversity (Mila et al., 2000).

Despite the different environmental agents driving changes in species distributions (permafrost and ice-sheets versus aridity and deserts), we might expect some similarities between temperate and boreal taxa, and their arid-zone counterparts, in their genetic response to major climatic change. Assuming that the expansion of arid systems was unfavorable for mesic biota, the distribution of mesic taxa would have been fragmented as these organisms retreated into moister pockets that typically occur in areas of higher elevation and topographic complexity. Indeed, rocky ranges in present-day arid zones often harbor discrete genealogical lineages (e.g. Smit et al., 2007). However, in contrast to

Northern Hemisphere refugial scenarios, where expansion and contraction of species distributions occurred regularly throughout the last three million years, the onset and development of arid conditions during the Miocene eventuated in increasing and permanent aridification, potentially isolating mesic taxa in refugia for extremely long periods of time. We would therefore predict that populations restricted to mountain refugia in arid zones, and thus unaffected by severe aridification, should show high levels of genetic diversity and deep phylogeographic structure among disjunct refugia, with deep coalescence histories resulting from a large, constant population size and many millions of years in isolation. For arid-zone taxa distributed throughout the low-lying desert regions, we would expect populations to be less diverse than their rocky relatives due to the geologically young nature of the desert biomes. Furthermore, in regions most affected by severe Plio/Pleistocene aridification and the advancement of sand dunes, we expect populations to have very shallow coalescence histories due to recent bottlenecks, as well as strong signatures of population expansion as arid-adapted taxa expanded their distributions with the opening of new desert biomes (Fujita et al., 2010).

These predictions are intuitively obvious, but they remain poorly tested because few examples in the literature incorporate sampling of widespread taxa that would allow the direct genetic comparison of taxa distributed across both mountain and desert

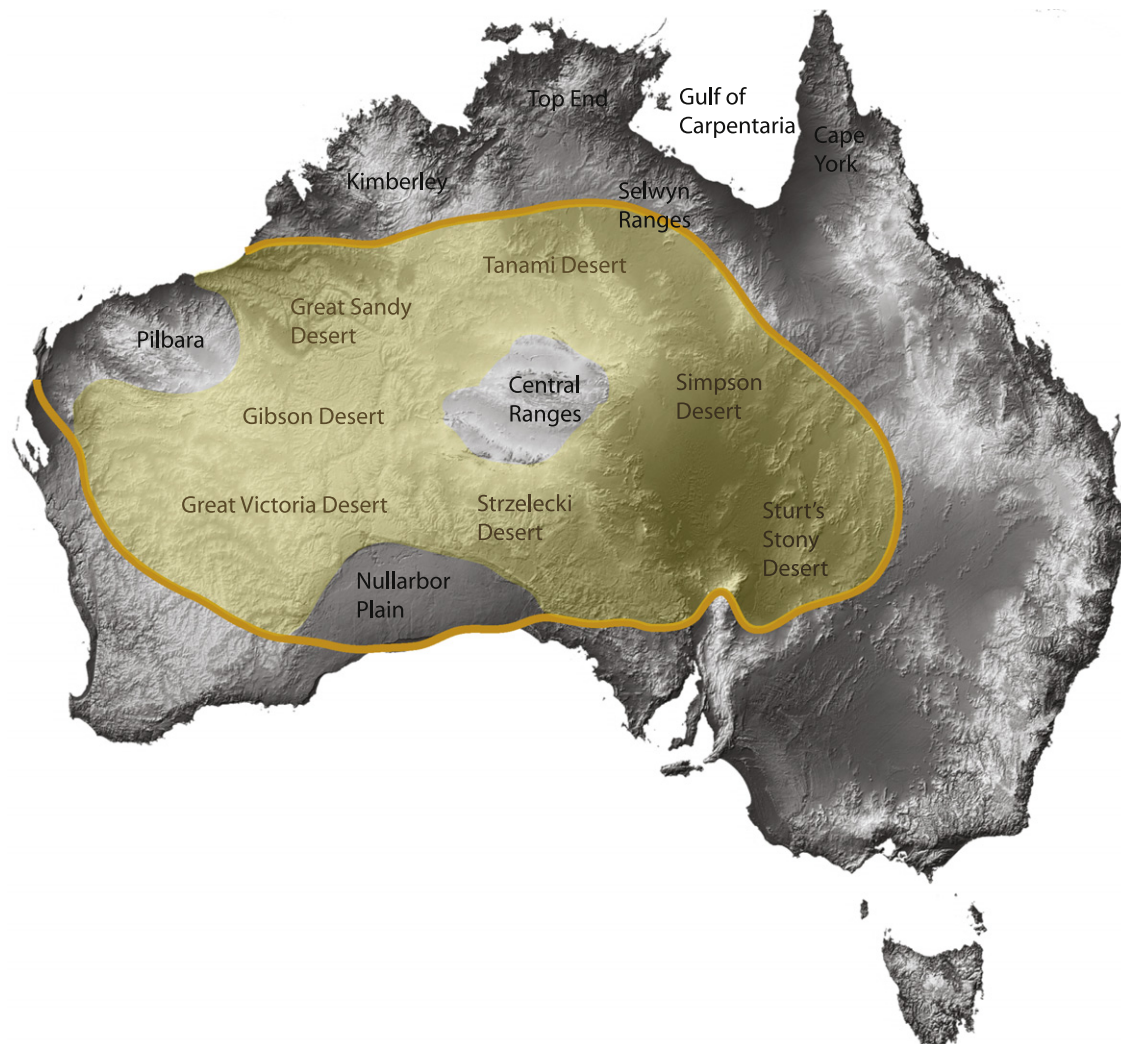


Fig. 1. Elevation map of Australia showing the extent of the arid zone (solid line), the distribution of sand deserts (shaded yellow) and major geographical features mentioned in the text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

biomes. Here we use a combination of new and published genetic data from three wide-ranging gekkonid radiations that span the vast arid zone of Australia, a biome larger than the area of Europe, to test hypotheses on refugial isolation and arid-zone diversification. This region presents an ideal system because it contains a number of ancient, stable, topographically complex areas that are isolated by a continuous expanse of low-lying sand deserts thought to have existed for less than one million years (Fig. 1; Fujioka et al., 2009). We analyse large mitochondrial DNA (mtDNA) datasets to evaluate the genetic patterns and evolutionary histories of mountain- and desert-dwelling clades in response to changing climates since the Miocene, specifically to address the following questions. (1) Do mountain ranges harbor more diversity than the surrounding deserts? (2) Is the diversity in mountain ranges older than in the deserts? We consider our results for these questions, along with the inferred demographic history, in light of the climatic and landscape history of the Australian arid zone to address a third question. (3) How did populations respond to increasing aridity and more severe glacial cycles of the mid-Pleistocene? Our results imply very different evolutionary outcomes for taxa in mountain versus desert landscapes, and suggest the deepening aridity of the Pleistocene had a profound influence on the genetic architecture of desert clades.

2. Methods

2.1. Sampling and sequencing

We generated large mtDNA datasets from three gekkonid radiations, providing us with three independent estimates of coalescent histories. Recent genetic sequencing of nuclear loci supports the major mtDNA phylogroups in each of the taxa (Pepper et al., 2006; Oliver et al., 2007; Fujita et al., 2010; Pepper et al., 2011). The gekkonine genus *Heteronotia* consists of three described species. *Heteronotia binoei* has the largest distribution and ecological range occurring over most of Australia, and previous work has uncovered numerous chromosomal and genetically divergent lineages (Moritz 1993, Fujita et al., 2010). While these studies recognize three “arid zone” lineages (CA6, SM6 and EA6), based on geographic distribution for the purpose of our study, we only consider genetically discrete groups within SM6 and EA6 (referred to as SM6 desert and EA6 desert, respectively) to be true desert lineages, because their distributions extend through the central and southern deserts. A further two species of *Heteronotia*, *H. spelea* and *H. planiceps*, are rock specialists restricted to mountainous regions in western and central Australia. Previous genetic work on *H. spelea* showed that this species comprises two genetically divergent and geographically disjunct populations, herein referred to as Pilbara *H. spelea* and Central *H. spelea* (Pepper et al., 2011), while *H. planiceps* likely represents a species complex (Pepper et al., 2011). The diplodactyline genus *Lucasium* comprises a number of species largely confined to the arid zone. Of these, *L. stenodactylum* has the broadest distribution, which spans the sandy and stony substrates of the arid zone and also extends into the Kimberley and Northern Territory. The monotypic *Rhynchoedura* is closely related to *Lucasium* (Pepper et al., 2006; Oliver et al., 2007) and is distributed throughout arid and semi-arid Australia on sands and heavy clays (Storr et al., 1990).

We attempted to maximize the geographic coverage of the target species. For the majority of species our coverage represents the best possible sampling from existing collections of the Western Australian Museum, the South Australian Museum, the Australian Museum, and the Museum of Vertebrate Zoology at the University of California, Berkeley (Fig. 2). Our datasets consist of 179 *Heteronotia*, 220 *Lucasium stenodactylum*, and 189 *Rhynchoedura ornata*

individuals. Our *Heteronotia* dataset consists of previously published sequences from the three described species, *H. binoei*, *H. spelea*, and *H. planiceps* (Fujita et al., 2010; Pepper et al., 2011). Details of all the chromosome races of *H. binoei* can be found in Fujita et al., (2010). The majority of our *L. stenodactylum* samples represent previously published material (Pepper et al., 2006; Pepper et al., 2008); however, we extended our sampling, particularly in South Australia, to provide a clearer picture of the distribution of genetic diversity across the southern arid zone. Our *Rhynchoedura* dataset largely consists of sequences generated for this study. Museum accession numbers and collection localities are given in Supplementary Table 1.

2.2. DNA sequencing and alignment

For all samples, a ~1200 bp fragment of the mitochondrial genome was targeted, extending from tRNA^{Met} through the entire protein-coding gene *nd2* (NADH dehydrogenase subunit II) and the genes encoding tRNA^{Trp}, tRNA^{Ala}, and tRNA^{Asp}. For details of PCR amplification and sequencing, including reactions and primer combinations, see Pepper et al. (2006) and Fujita et al. (2010). Each edited *nd2* alignment was 1038 base pairs long after removing the tRNAs. Alignment of the mitochondrial sequences was first performed automatically using the software MUSCLE (Edgar, 2004), then refined by eye in Se-AL (Rambaut, 2007). Protein-coding regions were translated into amino acid sequences and checked for internal stop codons and frame-shift mutations.

2.3. Diversity and demographic analyses

To infer past demographic patterns in response to aridification, we calculated nucleotide diversity and tested for population size change using the basic population genetic measurements of Tajima's *D* (Tajima, 1989) and Fu's *F_s* (Fu, 1997) as implemented in Arlequin v. 3.1.1. (Excoffier et al., 2005). Estimates and tests for significance were calculated for each population, corresponding to the phylogenetically distinct groups based on the mtDNA gene tree.

2.4. Bayesian phylogenetic and dating analysis

We used a Bayesian phylogenetic approach to co-estimate the phylogeny, demographic history, and divergence times for each of the study species. Estimation of the time-scale requires some form of calibrating information, which is difficult for our datasets because of the paucity of reliable fossil data for Gekkota. Therefore, we used a two-step approach to obtain estimates of the time-scale. In the first step, a sample of species of Gekkota was analysed in order to obtain estimates of divergence times. These age estimates were then used as secondary calibrations for the second step, in which analyses of demographic history were conducted.

2.4.1. Step 1: Estimation of divergence times

To estimate divergence times within Gekkota, we downloaded nucleotide sequences for nuclear *rag1* and mitochondrial *nd2* from Genbank for eight species, representing the major lineages within the infraorder (Supplementary Table 2). We also included new sequences of *Lucasium*, *Rhynchoedura*, and *Heteronotia*. For each of the three species *R. ornata*, *L. stenodactylum*, and *H. binoei*, we randomly selected representatives from the two most divergent lineages in order to ensure that the most recent common ancestor (MRCA) of each species was included in the tree. This allowed us to estimate the time to the MRCA (TMRCA) of each species in order to provide calibrating information for the next step of the analysis. To reduce the impact of mutational saturation in the analysis, the third codon sites of *nd2* were excluded from the analysis. The

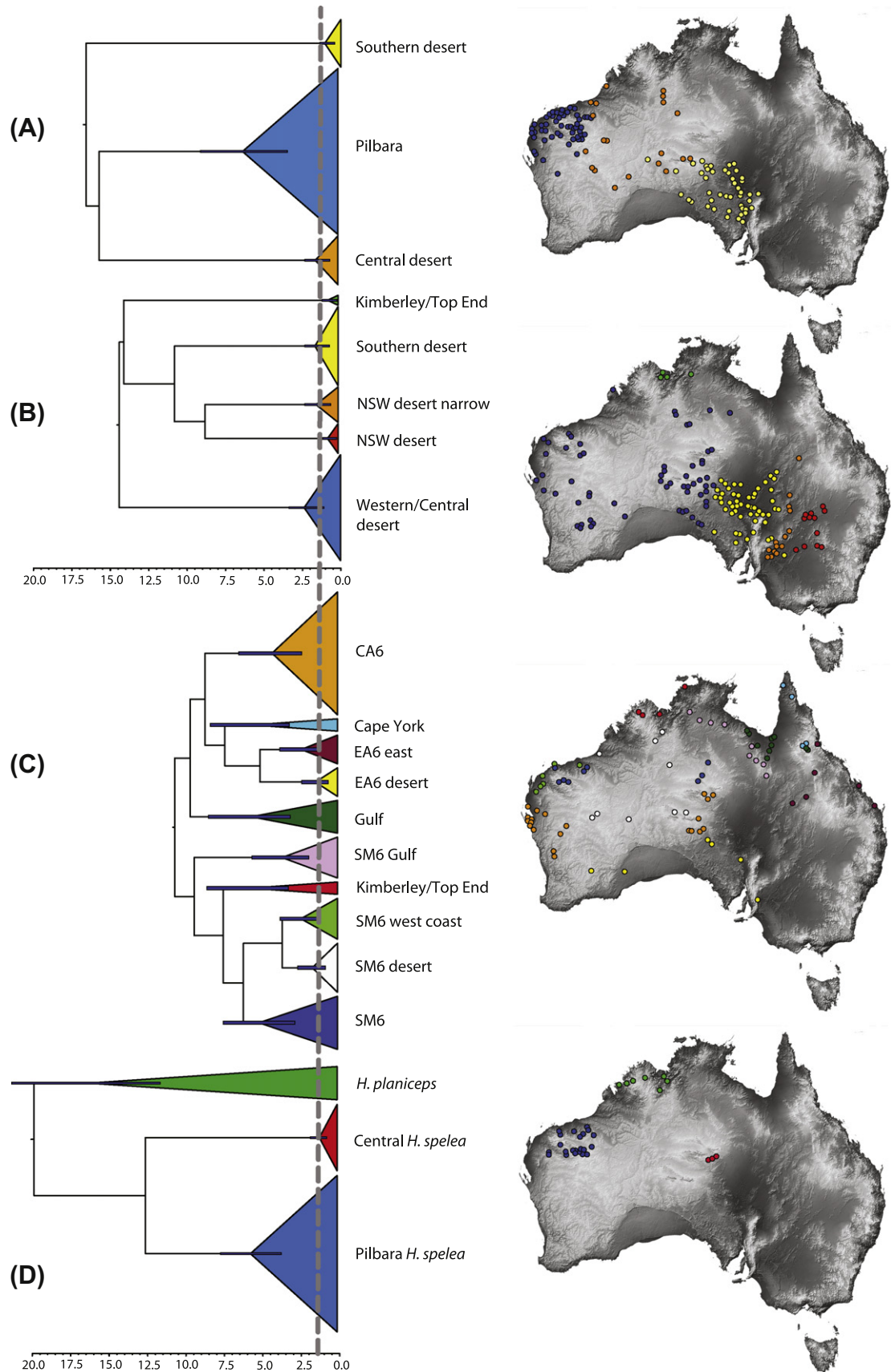


Fig. 2. Chronograms inferred using Bayesian molecular clocks in BEAST v.1.6 using only 3rd codon positions of mtDNA, alongside the distribution of each major clade of (A) *L. stenodactylum*, (B) *R. ornata*, (C) *H. binoei* and (D) *H. planiceps*/*H. spelea*. Localities shown on the maps are color-coded with their associated clade. Time axis in millions of years is indicated on the bottom. Nodal bars are 95% confidence intervals. Dashed gray line indicates a period of intense aridification during the Pleistocene (McLaren and Wallace 2010). For detailed gene trees showing all branching events, see Supplementary Figs. 1–4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resulting sequence alignment comprised 2784 bp (682 bp from *nd2*, 2102 bp from *rag1*) from 17 taxa.

The lack of multiple, reliable fossils within squamates, particularly geckos, limits the estimation of divergence times. Typically, phylogenetic studies of squamates using *nd2* have been calibrated using a substitution rate of 6.5×10^{-9} substitutions/site/year (error range 6.1×10^{-9} – 7.0×10^{-9}), originally obtained by Macey et al. (1998). However, assuming that a single rate applies to all reptiles and amphibians might be problematic. For example, there is evidence of substantial rate heterogeneity among reptilian lineages (e.g. Bromham, 2002; Eo and DeWoody, 2010). Macey et al. (1999) estimated a gecko-specific rate of 5.7×10^{-9} substitutions/site/year based on *Teratoscincus* geckos. However, this rate applies to species-level comparisons, whereas it is likely that a higher rate is more appropriate for intraspecific analyses (Ho et al., 2005; Ho and Larson, 2006). A number of Australian gekkonid phylogenies have been calibrated using a Miocene pygopodid fossil (Hutchinson, 1998) (e.g. Jennings et al., 2003; Oliver et al., 2007). While a recent study incorporating molecular and morphological data found evidence corroborating the previously hypothesized phylogenetic placement of this fossil (Lee et al., 2009), some uncertainty remains as to its phylogenetic placement in the tree of extant species, therefore we have not used this fossil in our dating analyses.

The phylogeny and divergence times were co-estimated in a Bayesian phylogenetic framework using BEAST 1.5.4 (Drummond and Rambaut, 2007). The two data partitions, *nd2* and *rag1*, were assigned separate substitution models, selected by comparing values of the Bayesian information criterion as implemented in Modelgenerator (Keane et al., 2006). A Yule speciation prior was used for the tree topology and node times. To allow for rate variation among lineages, the phylogenetic analysis was performed using an uncorrelated lognormal relaxed clock (Drummond et al., 2006). The analysis was repeated using a strict clock after the posterior distribution of the coefficient of variation of rates, which measures the degree of departure from a strict clock, was found to include a value of zero.

The fossil *Cretaceogekko burmae* (Arnold and Poinar, 2008), which is thought to represent the oldest unequivocal gecko and has been dated at 97–110 million years (Ma) old, was used to calibrate the root of the Gekkota tree. To allow for uncertainty in the age of the calibration, the prior age of the root was assumed to be exponentially distributed with a minimum bound of 100 million years (Ma) and with 95% of the prior density between 100 and 150 Ma.

Posterior distributions of parameters, including divergence times, were estimated via Markov Chain Monte Carlo (MCMC) sampling. Samples were drawn every 2000 steps over a total of 20,000,000 MCMC steps, with the first 10% discarded as burn-in. Acceptable convergence to the stationary distribution was checked by inspecting the posterior samples using the diagnostic software Tracer v1.5 (Drummond and Rambaut, 2007). Effective sample sizes were >2000 for all parameters, indicating sufficient sampling from the posterior.

2.4.2. Step 2: Estimation of demographic history and divergence times

The second step of our dating analysis utilized our large-scale *nd2* datasets to obtain age estimates for the TMRCA of the major

lineages. The total dataset was divided into four subsets which were analysed separately: (i) *Rhynchoedura*, (ii) *Lucasium*, (iii) *H. binoei*, and (iv) *H. spelea* and *H. planiceps*.

It has been suggested that deep, interspecific calibrations are not appropriate for estimating divergence times among conspecific individuals (Ho and Larson, 2006). This is because rates appear to be time-dependent, whereby higher rates are observed over smaller phylogenetic depths or shorter time-scales (Ho et al., 2005). This effect has been ascribed to a number of potential factors, with incomplete purifying selection suggested as one of the major contributors (Subramanian et al., 2009). In support of this hypothesis, third codon sites in vertebrate mtDNA, which are presumably subject to a lesser degree of selective constraint than the first and second codon sites, have been shown to be relatively clocklike across more recent time-scales (Subramanian et al., 2009). For this reason, we used only third codon sites. For the sake of comparison, however, analyses were repeated using all three codon sites.

To estimate the best-fit model of nucleotide substitution for the four subsets of data, we used the Bayesian information criterion as implemented in Modelgenerator (Keane et al., 2006). The chosen models were TIM + G (*Rhynchoedura*), TrN + G (*Lucasium*), TIM + G (*H. binoei*) and TIM + G (*H. spelea* and *H. planiceps*). Models incorporating a proportion of invariant sites were disregarded because of the intraspecific nature of most of the datasets.

The date estimates from Step 1 (described above; Table 1) were used to calibrate the root of the gene tree for each species, with the calibration priors assumed to be normally distributed. For comparison, divergence times were estimated by specifying a normal prior distribution (mean 5.7×10^{-9} subs/site/year, standard deviation 2.9×10^{-10} subs/site/year) for the substitution rate based on the estimate by Macey et al. (1999).

3. Results and discussion

3.1. Mountain ranges harbor more diversity than the surrounding deserts

Phylogenetic and population genetic analyses for all three gecko groups broadly support the hypothesis that genetic diversity is greatest in the topographically complex parts of the continent, whereas clades occupying the low-lying desert interior have less genetic diversity. This pattern is the same as that recently documented in a series of examples in invertebrates (Garrick 2011). Clades distributed in mountain ranges are characterized by deep phylogenetic structure, while shallower structure characterizes desert clades (Fig. 2 and Suppl. Figs. 1–4). Levels of nucleotide diversity corroborate this result, showing that saxicolous clades typically have higher nucleotide diversity than desert clades (Table 2). The geographic distributions of clades lend further support to this pattern, where it is apparent that clades with high nucleotide diversity often occur across smaller geographic areas, whereas desert clades displaying substantially lower nucleotide diversity often are distributed over vast geographic areas (Fig. 2). This result is exemplified in our data from *Lucasium*, where populations distributed throughout the geographically vast southern and central deserts have between 5 and 18 times less nucleotide diversity than populations in the rocky Pilbara region (Table 2).

Table 1
Posterior root heights with 95% Highest Posterior Density (HPD) estimates in brackets for clades based on a Bayesian phylogenetic analysis of mitochondrial *nd2* and nuclear *rag1*. Dates are presented in millions of years (Myr).

Clade	Root height at all sites (Myr)	Root height at third codon sites (Myr)	Root height using Macey et al. (1999) rate (Myr)
<i>L. stenodactylum</i>	16.1 (9.7–22.7)	16.3 (9.9–22.5)	19.9 (15.9–24.0)
<i>R. ornata</i>	14.2 (8.2–20.1)	14.2 (8.4–20.4)	18.5 (15.2–21.8)
<i>H. binoei</i>	10.6 (6.5–14.7)	10.6 (6.6–14.7)	26.5 (22.6–30.5)
<i>H. spelea/H. planiceps</i>	19.1 (14.9–23.6)	19.7 (15.1–24.4)	32.1 (26.9–37.6)

Table 2Diversity and demographic summary statistics. Bold labels indicate desert clades. *p* values in italics indicate significance at the 5% level.

Clade	Tajima's <i>D</i>	<i>p</i> value	Fu's <i>F_s</i>	<i>p</i> value	Nucleotide diversity
<i>R. ornata</i> NSW desert	−1.16572	0.11500	−5.50464	0.00800	0.004101±0.002364
<i>R. ornata</i> NSW desert narrow	−1.41117	0.05500	−8.43479	0.00200	0.006724±0.003637
<i>R. ornata</i> Southern desert	−1.74616	0.01600	−24.51409	0.00001	0.010609±0.005428
<i>R. ornata</i> Western/Central desert	−1.68004	0.01900	−24.15092	0.00001	0.015753±0.007864
<i>R. ornata</i> Kimberley/Top End	0.37704	0.68700	−0.94519	0.23100	0.006842±0.004094
<i>L. stenodactylum</i> Pilbara	−0.38172	0.40700	−23.98100	0.00200	0.040324±0.019507
<i>L. stenodactylum</i> Central desert	−1.75332	0.02100	−17.96309	0.00001	0.007876±0.004153
<i>L. stenodactylum</i> Southern desert	−2.54989	0.00001	−26.60136	0.00001	0.002442±0.001494
<i>H. planiceps</i>	0.11404	0.55900	1.48798	0.46700	0.15009±0.082259
<i>H. spelea</i> Central	0.84724	0.85100	1.30056	0.72400	0.022372±0.011701
<i>H. spelea</i> Pilbara	0.89232	0.85200	−0.13347	0.47600	0.079746±0.039113
<i>H. binoei</i> CA6	0.31325	0.71800	−1.14687	0.35600	0.046824±0.023180
<i>H. binoei</i> EA6 desert	0.00794	0.52200	2.49120	0.85800	0.020765±0.011701
<i>H. binoei</i> EA6 east	0.02204	0.51400	−0.04408	0.30100	0.034912±0.019421
<i>H. binoei</i> SM6	1.24583	0.92500	0.92570	0.52300	0.062205±0.032084
<i>H. binoei</i> SM6 west coast	0.58737	0.78100	−1.41948	0.14900	0.016877±0.009022
<i>H. binoei</i> SM6 desert	−0.30354	0.42900	−3.70571	0.03700	0.030824±0.016462
<i>H. binoei</i> SM6 gulf	0.70989	0.79000	0.71495	0.54600	0.040476±0.021504
<i>H. binoei</i> Kimberley/Top End	0.29321	0.74400	2.46431	0.54000	0.075759±0.049851
<i>H. binoei</i> Gulf	0.89029	0.84600	0.22664	0.33600	0.061673±0.033415
<i>H. binoei</i> Cape York	0.90317	0.80500	2.65317	0.53800	0.091754±0.060291

Similarly, whereas the SM6 desert *H. binoei* clade has nucleotide diversity on par or higher than SM6 west coast and EA6 east (0.03 versus 0.02 and 0.03, respectively; Table 2), the geographic distribution of SM6 desert spans the vast region of the northern and central deserts (Fig. 2) and covers a substantially larger area than either SM6 west coast or EA6 east.

Our results for Kimberley/Top End *R. ornata* are at odds with the high diversity typically seen in other Monsoon Tropic reptile taxa (i.e. Melville et al., 2010; Oliver et al., 2010; Fujita et al., 2010; Pepper et al., 2011). Although there are records of this species from elsewhere in the Kimberley (Online Zoological Collections of Australian Museums database), our sampling for this clade (represented by eight sequences) only includes individuals from low-lying regions in the Kimberley gulf, as well as an individual from Victoria River in Gregory National Park. The ecology of *R. ornata* and its adaptation to sand/clay substrates suggests that this taxon is unlikely to be found on rocky uplands, and might explain the low diversity of the Kimberley/Top End clade.

The pattern of genetic diversity within and among gecko lineages in our study is broadly consistent with phylogeographic studies of Northern Hemisphere taxa distributed across mountain and lowland regions. High nucleotide diversity within, and strong phylogeographic structure between, gecko clades in isolated mountain ranges is indicative of long-term isolation and diversification in refugia (Hewitt, 1999). This result is expected for our data, given the unlikely potential for repeated geographical contact between disjunct refugia in the Australian arid zone following intense aridification of the Plio/Pleistocene and the subsequent development of the intervening sand deserts. The low nucleotide diversity displayed by gecko clades that occupy the desert regions of the continent is indicative of rapid expansion of arid-adapted lineages into large areas of suitable territory (Castoe et al., 2007), and is consistent with other studies of Australian arid-zone vertebrates that show little genetic differentiation across the arid zone (Kuch et al., 2005; Joseph and Wilke, 2007). This pattern is expected because of the recent nature of severe aridity, particularly in the interior lowlands, and the development of widespread sand deserts <1 Ma.

3.2. Diversity in mountain ranges is older than in deserts

Our dating estimates using all codon sites compared to only third codon sites were very similar (Tables 1 and 3), perhaps

because of the long time-scale of evolution in each taxon. In contrast, estimates using the Macey et al. (1999) substitution rate were typically older, which is most obvious in the *Heteronotia* taxa (Tables 1 and 3). Regardless of which dating method was used, an emerging pattern from our data is that clades inhabiting rocky ranges are generally older than desert clades. Our TMRCA estimates for the saxicolous clades (Pilbara *H. spelea* and Pilbara *L. stenodactylum*, *H. planiceps* and Kimberley/Top End/Gulf/Cape York *H. binoei*) date to the mid-late Miocene. In contrast, our TMRCA estimates for the desert clades (Central and Southern desert *L. stenodactylum*, Western/Central desert, Southern desert, NSW desert and NSW desert narrow *Rhynchoedura*, and SM6 desert and EA6 desert *H. binoei*) all post-date the Pleistocene. The *H. binoei* clades EA6 east and SM6 west coast also have TMRCA estimates that date to the Pleistocene, however, in the case of SM6 west coast, this clade is distributed in the low-lying coastal zone in the western Pilbara, and is not associated with upland ranges. The young age of EA6 east distributed throughout Queensland is unexpected and does not corroborate our predictions.

Rocky ranges located on the periphery of the Australian continent, such as the Pilbara, Kimberley, and Top End, were protected from climatic extremes due to their close proximity to the coast (Byrne et al., 2008). Our phylogenetic and dating analyses support an ancient evolutionary history of saxicolous clades in these regions, consistent with the persistence of these taxa since the late Miocene (Suppl. Figs. 1–4). However, the Central *H. spelea* clade in the rocky ranges of central Australia is estimated to have a much younger MRCA of <1 Ma. Previous studies on the climate and geomorphological history of central Australia have suggested that because this region is completely isolated by deserts, conditions during past glacial cycles were likely to have been more severe, with temperatures much colder than at present (Miller et al., 1997). This hyper-arid and unstable climatic history is thought to have repeatedly extinguished narrowly endemic taxa in central Australia, particularly during the punctuated Pleistocene arid cycles (Crisp et al., 2001), and offers an explanation for the very young age of this clade relative to the others in the genus (Pepper et al., 2011).

The opening of desert biomes and the distribution and movement of sand deserts are known to have profoundly influenced diversification patterns and distributions of arid-zone taxa (Scott et al., 2004; Melville et al., 2009; Fujita et al., 2010). In all of our inferred genealogies, the MRCA estimates of clades distributed

Table 3
Mean TMRCA estimates and associated 95% Highest Posterior Density (HPD) estimates for major clades from a Bayesian phylogenetic analysis of third codon sites of mitochondrial *nd2*. Dates are presented in millions of years (Myr). Bold labels indicate desert clades.

Clade	3rd codon sites mean (Myr)	All codon sites mean (Myr)	Macey et al. (1999) rate mean (Myr)
<i>R. ornata</i> NSW desert	0.69 (0.26–1.16)	0.69 (0.31–1.11)	0.92 (0.58–1.33)
<i>R. ornata</i> NSW desert narrow	1.43 (0.63–2.33)	1.27 (0.60–2.00)	1.65 (1.07–2.33)
<i>R. ornata</i> Southern desert	1.50 (0.73–2.32)	1.31 (0.67–2.03)	1.72 (1.22–2.30)
<i>R. ornata</i> Western/Central desert	2.20 (1.08–3.36)	1.97 (1.05–3.02)	2.59 (1.98–3.28)
<i>R. ornata</i> Kimberley	0.68 (0.24–1.20)	0.65 (0.28–1.08)	0.87 (0.48–1.28)
<i>L. stenodactylum</i> Pilbara	6.17 (3.45–9.07)	4.70 (2.53–6.94)	5.87 (4.76–7.05)
<i>L. stenodactylum</i> Central desert	1.47 (0.72–2.33)	1.14 (0.57–1.83)	1.43 (0.99–1.95)
<i>L. stenodactylum</i> Southern desert	0.85 (0.40–1.36)	0.62 (0.30–0.99)	0.77 (0.49–1.06)
<i>H. planiceps</i>	16.2 (11.5–21.2)	18.0 (13.8–22.5)	30.2 (25.2–35.7)
<i>H. spelea</i> Central	1.15 (0.68–1.74)	2.12 (1.38–2.92)	3.57 (2.54–4.68)
<i>H. spelea</i> Pilbara	5.64 (3.63–7.58)	7.90 (5.77–9.98)	13.3 (10.8–15.6)
<i>H. binoei</i> CA6	4.26 (2.33–6.44)	3.73 (2.11–5.27)	9.30 (7.52–11.3)
<i>H. binoei</i> EA6 desert	1.43 (0.59–2.33)	1.15 (0.63–1.74)	2.86 (2.05–3.72)
<i>H. binoei</i> EA6 east	2.36 (1.05–3.75)	2.54 (1.44–3.70)	6.36 (4.79–7.91)
<i>H. binoei</i> SM6	5.14 (2.90–7.62)	4.10 (2.29–5.81)	10.3 (8.05–12.6)
<i>H. binoei</i> SM6 west coast	2.38 (1.10–3.74)	2.32 (1.28–3.36)	5.79 (4.47–7.32)
<i>H. binoei</i> SM6 desert	1.64 (0.79–2.59)	1.12 (0.57–1.66)	2.79 (1.95–3.75)
<i>H. binoei</i> SM6 gulf	3.61 (1.86–5.57)	3.34 (1.84–4.78)	8.33 (6.33–10.4)
<i>H. binoei</i> Kimberley/Top End	5.77 (3.19–8.52)	4.76 (2.80–6.80)	11.9 (9.31–14.4)
<i>H. binoei</i> Gulf	5.63 (3.07–8.40)	5.14 (3.05–7.42)	12.8 (9.92–15.9)
<i>H. binoei</i> Cape York	5.54 (3.13–8.28)	5.62 (3.42–8.08)	14.0 (11.4–17.0)

throughout the Australian deserts correspond to the initiation of severe aridification across the Australian arid zone. Long-standing debate centers on the timing and pace of diversification in various groups of Northern Hemisphere biota (Avise et al., 1998; Lovette, 2005) and the role of Pleistocene events in promoting speciation. Our results clearly demonstrate that while species-level divergences substantially pre-date the Pleistocene, the dynamic climate fluctuations of this time period had a marked impact on the genetic architecture of arid-zone desert clades.

3.3. Rock and desert populations respond differently to the severe glacial cycles of the Pleistocene

Neutrality statistics of Fu's F_s and Tajima's D for the majority of saxicolous clades suggest no deviation from neutral expectations, which can be interpreted demographically as stable populations in mutation-drift equilibrium (Table 2). In contrast, analysis of all desert clades of *Rhynchoedura* and *L. stenodactylum* revealed significant negative estimates of F_s and D (Table 2), consistent with a signature of contiguous range expansion. In some clades the statistical inference of expansion was not significant for Tajima's D (*Rhynchoedura* NSW desert, NSW desert narrow), but moderately large, negative F_s values were significant (Table 2). These results might be due to the greater power of Fu's F_s to detect population growth (Ramos-Onsins and Rozas, 2002). Interestingly, Pilbara *L. stenodactylum* clade shows a significant and large negative estimate for Fu's F_s (-23.98 , $p < 0.002$), that also is not reflected in significant estimates of Tajima's D (-0.38 , $p < 0.40$). Substantial landscape heterogeneity exists within the Pilbara region, with the southern Pilbara dominated by the rocky uplands of the Hamersley Range, while the northern Pilbara also comprises weathered lowlands. Intra-regional instability during severe climate cycles could have affected the population dynamics of Pilbara *L. stenodactylum* and may have contributed to the signal of population expansion evidenced in this clade.

Historical population expansions over large continental areas following Pleistocene glacial maxima are thought to explain the lack of variation and phylogeographic structure in Northern Hemisphere taxa (Hewitt, 1999), with the timing of these expansions often coinciding with the most recent deglaciation event ~ 20 ka ago (e.g. Mila et al., 2000). A number of studies have documented recent range expansions of widespread and highly vagile organisms

in the African dry tropics (Van Hooft et al., 2002; Lorenzen et al., 2008) and across the Australian arid zone (Joseph and Wilke, 2007; Neaves et al., 2009) following a peak of Pleistocene aridity and widespread desertification. Our results illustrate the similarity in response of substantially less vagile biota to the severity of Pleistocene arid conditions.

3.4. Synthesis

Palynological and geological data demonstrate that aridification was prevalent on most continents from the Miocene (Flower and Kennett, 1994), and the initiation and spread of xeric conditions is thought to have had a profound effect on biotic diversification globally (van Dam, 2006; Byrne et al., 2008; Melville et al., 2009). In addition, aridification of desert regions became increasingly severe in the middle Pleistocene in response to major global climatic variations (Clark et al. 2006), but few studies have examined the genetic consequences of this turbulent climatic period for arid-zone taxa. The mtDNA genetic patterns and associated ages of lineages and clades in the geckos examined here are consistent with fragmentation, isolation, and reduction in response to the deepening aridification of the Australian continent. While our interpretation rests on results inferred from a single locus, we have found largely congruent mtDNA patterns across three independent gecko radiations, and so have estimates of three independent coalescent histories. Collectively, our data provide novel insights into the evolutionary histories of taxa that evolved in concert with wide-scale aridification and desert formation.

The geographic distributions of *L. stenodactylum*, *Heteronotia* and *Rhynchoedura* are partitioned within the vast arid zone of Australia, including the rocky ranges in western, northern, and central Australia (Cogger, 2000). Deep phylogeographic structuring exists in all taxa, reflecting long-term isolation of clades since the Mid-Miocene (~ 15 Ma). This timing suggests that initial diversification was likely driven by the onset of dry conditions and associated contraction of mesic forest early in the history of aridification on the Australian continent (Martin, 2006). Similar divergence-time estimates have been inferred for arid-zone reptiles in central Asia (Melville et al., 2009), Africa (Scott et al., 2004) and the southwestern United States (Devitt, 2006), corresponding to the initiation of xeric conditions in these regions. Our divergence dates also are consistent with a number of previous studies that included arid

and semi-arid reptiles in Australia (reviewed in Byrne et al., 2008), as well as the timing of species-level diversification in Australian mesic biomes (Schneider et al., 1998). Our data add to the mounting body of molecular evidence indicating that widespread landscape and climate changes associated with the initiation of aridification in the Miocene had a profound influence on the higher-level diversification of the Australian biota (Crisp et al., 2004).

The high nucleotide diversity recovered in clades in the mountain ranges might be the result of a larger and more stable historical population size (Avice, 2000). If we assume that mesic taxa were unable to move out of refugia following severe aridification of the Plio/Pleistocene, or possibly even earlier depending on the sensitivity and thermal tolerances of the organism, diversity has been sustained in these mountainous pockets for extremely long periods of time. While tectonic uplift and mountain building has been strongly linked to recent biotic diversification on many continents (Trewick and Wallis, 2001; Che et al., 2010), the mountain ranges of central and Western Australia have been tectonically stable for more than 100 million years (van Kranendonk et al., 2002), and cannot account for the generation of such diversity in these regions. However, landscape heterogeneity, such as variation in geology, topography and soil diversity, also is known to be important for promoting genetic diversity and speciation (Fine et al., 2005; Rosenblum, 2006; Garrick, 2011). The topographic and spatial diversity of rock types and soil lithologies in the rocky uplands of central, northern and western Australia is linked to the ancient and unique geological history of the Australian craton exposed in these uplifted mountain regions (Wasson, 1982). The associated variations in microclimate and vegetation may favor habitat specialization and the emergence and maintenance of diversification (Alvarez et al., 2009). Much like mesic taxa in the well-studied northeast Queensland rainforest (Schneider et al., 1998), our results clearly demonstrate that persistence of biota as aridity oscillated and intensified in the Plio/Pleistocene was maintained in climatically stable mountain refugia in and adjacent to the central arid zone, and that diversity in these regions was sustained through the Pleistocene by topographic and habitat complexity.

For taxa distributed throughout lowland areas in Australia's inland deserts, our results reflect a highly dynamic evolutionary history, which is expected given that these regions were most affected by intensifying aridification. While a number of molecular studies support intraspecific diversification during the Pleistocene in the Australian arid zone (reviewed in Byrne et al., 2008), our results provide further insights and suggest that the severe climate change at the onset of this time period likely erased much of the genetic diversity of taxa distributed in the central Australian lowlands prior to the development of the inland sand deserts. On the Australian continent, evidence for a major shift in climate regime 1.4–1.5 Ma has been recorded in sediments from a palaeolake in the southeast (McLaren and Wallace, 2010), indicating a major step in the trend towards severe aridification on a local scale. Furthermore, dating of desert dunes in central Australia suggests increasing amplitude of aridity and the formation of major inland sand deserts around 1 Ma (Fujioka et al., 2009). The majority of desert clades in our study have MRCA age estimates younger than 1.5 Ma, in line with the timing of these historical climate and landscape changes (Fig. 2 and Suppl. Figs. 1–4). The repeated pattern of low mtDNA variation and large, negative F_s values in widespread desert clades is consistent with expectations of recent population expansions in desert-dwelling taxa. Although we cannot entirely rule out the possibility of a selective sweep to explain the observed pattern of genetic variation, its recurrence in multiple, co-distributed clades suggest this explanation is less likely. *Rhynchoedura* and *L. stenodactylum* are likely to have inhabited these lowland regions following some level of adaptation to xeric environments since the mid-late Miocene. We interpret our results as evidence

of substantial population bottlenecks followed by recent expansion of desert clades in response to relative climatic amelioration as well as adaptation to sand desert environments. In contrast, ancestral habitat reconstructions for *Heteronotia* support a mesic origin (Fujita et al., 2010). Therefore, it seems more likely that the arid zone *H. binoei* clades have only recently occupied the central Australian lowlands, and expanded their ranges into the arid biome following the spread of xeric habitats in the Plio/Pleistocene. Diversity in these *H. binoei* clades was probably sustained in refugia outside of the central arid zone, before the desert clades expanded their distributions across the sandplains concomitant with the spread of the dunes.

Our molecular data provide novel insights into the genetic consequences of aridification for taxa in arid biomes. The dynamics of biological diversification occur in myriad different ways, and often are tied to specific geological and ecological histories of distinct lineages. However, broad-scale climate fluctuations, such as the global glacial cycles of the Pleistocene, leave their signatures on biotic diversification across collections of taxa (Hewitt, 1999; Moritz et al., 2000; Schneider et al., 1998). Despite differences in the actual details of climate and geological changes that occurred within and among regions and biomes, similar patterns emerge. In the Northern Hemisphere, this is evident from glaciations and the movement of continental ice sheets. In Southern Hemisphere arid zones, as demonstrated by the comparative phylogeography in this study, it is driven by aridification.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jympev.2011.08.009.

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