Evolutionary speed limited by water in arid Australia

Xavier Goldie^{1,*}, Len Gillman², Mike Crisp³ and Shane Wright¹

¹*School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland 1020, New Zealand*

²*School of Applied Science, Auckland University of Technology, Private Bag 92006,*

Auckland 1020, New Zealand

³*Research School of Biology, The Australian National University, Canberra, Australian Capital Territory 0200, Australia*

The covariation of biodiversity with climate is a fundamental pattern in nature. However, despite the ubiquity of this relationship, a consensus on the ultimate cause remains elusive. The evolutionary speed hypothesis posits direct mechanistic links between ambient temperature, the tempo of micro-evolution and, ultimately, species richness. Previous research has demonstrated faster rates of molecular evolution in warmer climates for a broad range of poikilothermic and homeothermic organisms, in both terrestrial and aquatic environments. In terrestrial systems, species richness increases with both temperature and water availability and the interaction of those terms: productivity. However, the influence of water availability as an independent variable on micro-evolutionary processes has not been examined previously. Here, using methodology that limits the potentially confounding role of cladogenetic and demographic processes, we report, to our knowledge, the first evidence that woody plants living in the arid Australian Outback are evolving more slowly than related species growing at similar latitudes in moist habitats on the mesic continental margins. These results support a modified evolutionary speed explanation for the relationship between the water-energy balance and plant diversity patterns.

Keywords: productivity; molecular evolution; species richness; generation time; water-energy dynamics

1. INTRODUCTION

Explaining the relationships between broad-scale variation in biodiversity and climate is a fundamental challenge at the nexus of ecology, evolutionary biology and biogeography (Currie *et al.* 2004). The most recognized global pattern in biodiversity is the trend of increasing taxonomic richness with decreasing latitude, exhibited by almost all major groups of terrestrial and aquatic organisms from plants and vertebrates to micro-organisms (Guernier *et al.* 2004; Hillebrand 2004; Pommier *et al.* 2007). Many hypotheses based on ecological (Wright *et al.* 1993), evolutionary (Rohde 1992; Allen *et al.* 2006; Jablonski *et al.* 2006), historical (Rosenzweig 1995; Wiens & Donoghue 2004; Stevens 2006), or geometric (Colwell & Lees 2000) processes have been proposed to explain these patterns, with little consensus emerging on which might be of primary importance.

Several hypotheses focus on potential latitudinal variation in net rates of diversification as a key mechanism behind the associated biodiversity gradients (Mittelbach *et al.* 2007). Thus, speciation and extinction rates are proposed to vary across latitudes, with the resultant accumulation of biodiversity being greater in tropical climates (Rohde 1992). One potential mechanism to explain these evolutionary disparities—the evolutionary speed hypothesis (Rohde 1992)—is receiving increasing attention. Its central component is that there is a direct

relationship between the higher energetic regime of the tropics and diversification, whereby higher temperatures result in a commensurate increase in metabolic activity, thus generating more mutations per unit time and simultaneously shortening generation times. Acting in tandem, these influences are proposed to increase both rates of DNA evolution and rates of positive selection, thereby leading to greater rates of cladogenesis and species accumulation.

Several lines of evidence provide support for the evolutionary speed hypothesis. There is increasing phylogenetic and palaeontological evidence that both net diversification rates and speciation rates have been faster at lower latitudes (Cardillo *et al.* 2005; Jablonski *et al.* 2006). A contrary pattern has also been proposed (Weir & Schluter 2007), although the assumptions used to derive those results have recently been empirically challenged (Gillman *et al.* 2009). Additionally, the rate of mutagenesis has been positively correlated with both metabolic rate (Martin & Palumbi 1993) and body temperature (Gillooly *et al.* 2005, 2007), while a negative relationship between generation time and substitution rate in plants has been demonstrated (Andreasen & Baldwin 2001; Smith & Donoghue 2008). Additionally, recent research has shown faster rates of molecular evolution in warmer climates among plants (Davies *et al.* 2004b; Wright *et al.* 2006; Gillman *et al.* in press), marine foraminifera (Allen *et al.* 2006), terrestrial mammals (Gillman *et al.* 2009) and amphibians (Wright *et al.* in press). An earlier test of differential rates of micro-evolution among birds produced equivocal results but with some (non-significant) evidence of faster rates at lower latitudes (Bromham & Cardillo 2003).

* Author for correspondence (xavier.goldie@anu.edu.au).

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Figure 1. Long-term average annual rainfall across continental Australia calculated for the period 1970–2000. Data courtesy of the Department for Climate Change, Canberra, Australia.

However, in terrestrial environments, both energy and water availability, and the interaction of those terms (the water-energy balance) have been found to be central to climate-based correlations with global biodiversity, including those not restricted to the latitudinal dimension (O'Brien 1998). Thus, species richness has been found to correlate independently and in combination with variables such as precipitation, actual evapotranspiration, net primary productivity and potential evapotranspiration (Currie & Paquin 1987; O'Brien 1993, 1998; Specht & Specht 1993; Hawkins *et al.* 2003, 2005). The water-energy balance refers to the dynamic relationship of energy and water (O'Brien 2000) in describing the resultant productivity of a system and its attendant biotic function. The relative availability of both energy and water might then control biological functions from cellular to systemic level, including factors influencing diversity. The latter is likely, given that a large proportion of the variation in species richness across a broad range of taxonomic groups is explained by variation in water-energy dynamics (e.g. Hawkins *et al.* 2003).

Therefore, the centrality of a water-energy balance to productivity and species richness underlies its importance to evaluations of hypotheses that attempt to explain those patterns (Gillman & Wright 2006). It has consequently been proposed that evolutionary speed should also be mediated by water availability, and not solely by ambient energy, if it is to provide a plausible explanation for global patterns of biodiversity (Gillman & Wright 2006, 2007; Wright *et al.* 2006).

Australia provides an ideal opportunity to test the concept of evolutionary speed with respect to the additional dimension of water availability, since this limiting factor has clearly played a central role in the evolutionary history of its biota (Crisp *et al.* 2004; Byrne *et al.* 2008). Water availability is very low in the continental centre and at the western-central and southern coastlines (the Eremaean Zone). By contrast, the eastern and

south-western margins of Australia are characterized by higher rainfall (figure 1) and elevated species richness (figure 2). Along these continua, there is a parallel gradient of increasing productivity, mediated almost entirely by the availability of water, towards the margins of the continent (figure 3).

Woody plant species in arid Australia are subjected to environmental regimes characterized by high potential evapotranspiration (a measure of thermal energy) and low actual evapotranspiration (a measure of climatic productivity potential), in conditions that are fundamentally controlled by a severe and sustained lack of water. These species must therefore endure substantial periods during which the use of abundant thermal energy for biological function is severely limited by water availability. Growth rates during these periods are likely to be substantially reduced, in conjunction with a putative drop in basic metabolic function, which may have a depressive effect on both rates of mutation and of nucleotide substitution (Gillooly *et al.* 2005). In addition, reduced growth rates could lengthen effective generation times and thus also retard the rate at which mutations spread to fixation (Smith & Donoghue 2008).

By contrast, species in more mesic environments experience much higher productivity regimes because of the greater availability of water and smaller disparities between potential and actual evapotranspiration (O'Brien 1998). Woody plants in wetter coastal environments are therefore subjected to more productive conditions that should, on average, facilitate greater biological activity and potentially higher metabolic rates, faster growth rates, shorter generation times, and higher rates of mutation and fixation. Using woody Australian plants as the experimental organisms, we tested whether the known relationship between latitudinal and elevational differences in productivity and molecular rate variation (Allen *et al.* 2006; Wright *et al.* 2006; Gillman *et al.* 2009) is replicated across a longitudinal gradient

Figure 2. Plant species richness for the Australian continent: species richness at 0.5° spatial grain calculated for 85 vascular plant families. Data courtesy of ANHAT, Canberra, Australia.

Figure 3. Long-term average annual productivity index, calculated for the period 1997–2000 at 1 km^2 spatial grain. Data courtesy of the Department of Climate Change, Canberra, Australia.

of productivity that is underpinned by the relative availability of water.

2. MATERIAL AND METHODS

(a) *Phylogenetically independent comparisons*

In this study, we tested the prediction that a water-mediated gradient in productivity would be reflected in differences in

nucleotide substitution rates in continental Australia. We did this by comparing substitution rates in the weakly selected (Van Nues *et al.* 1995) internal transcribed spacer (ITS) region of nuclear ribosomal DNA for species occurring in the arid zone, against those occupying the more mesic continental margins. This was done using maximum likelihood (ML) rate estimation for 30 phylogenetically independent pairs (table 1) of closely related woody species from across

Table 1. Ingroup and outgroup taxa, ML branch lengths and (mesic/arid) branch length ratios. (AP, arid parasite; RP, root parasite; CL, climber; SH, shrub; ST, small tree; MT, medium tree; LT, large tree. The ratios reported are not those used for the Wilcoxon sign-rank test (longest/shortest). References indicate existing phylogenetic literature used to guide in-group/out-group selection. PHYML indicates where PHYML was used with all available congeneric sequences either sequenced by us or in the public domain (GenBank) to determine optimum ingroup and outgroup species, with subsequent ingroup non-target taxon pruning and proximate outgroup selection for rate estimation with PAUP*. All GenBank accession details for all ingroup and outgroups; phylogenetic references and PHYML cladograms can be found in the electronic supplementary material.)

genus	arid species	length	mesic species	length	outgroups	ratio	habit
<i>Acacia</i> , Fabaceae: Mimosoideae, (PHYML)	<i>euthycarpa</i> ^a	0.01413	<i>prominiens</i> ^b	0.03454	<i>A. tetragonophylla</i> ^b ; <i>A. melanoxylon</i> ^b	2.444	ST/MT
	<i>loderi</i> ^b	0.02346	<i>maidenii</i> ^b	0.01048	<i>A. paradoxa</i> ^a ; <i>A. melanoxylon</i> ^b	0.447	MT/MT
	<i>oleifolius</i> ^b	0.02976	<i>tomentosus</i> ^b	0.01258	<i>A. excelsus</i> ^a ; <i>A. conatus</i> ^a ; <i>Pappea capensis</i> ^a	0.423	ST/ST
<i>Anyema</i> (Wilson & Calvin 2006; PHYML)	<i>maidenii</i> ^b	0.02027	<i>congener</i> ^b	0.01823	<i>A. cambagei</i> ^a ; <i>Dactylophora novae-guineae</i> ^a	0.899	AP/AP
<i>Atriplex</i> , Amaranthaceae, (PHYML)	<i>amnicola</i> ^a	0.01604	<i>cinerea</i> ^a	0.01424	<i>A. canescens</i> ^a ; <i>A. undulata</i> ^a ; <i>A. lentiformis</i> ^a ; <i>A. nummularia</i> ^a	0.888	SH/SH
	<i>elderiana</i> ^a	0.00449	<i>ilicifolia</i> ^a	0.01928	<i>B. quercifolia</i> ^a ; <i>B. nutans</i> ^a ; <i>B. menziesii</i> ^a ; <i>B. ashbyi</i> ^a ; <i>B. baxteri</i> ^a ; <i>Musgravea heterophylla</i> ^a	4.294	ST/MT
<i>Banksia</i> , Proteaceae, (PHYML)		0.00942	<i>rhomboidea</i> ^a	0.01447	<i>C. macleayana</i> ^c ; <i>C. sulcata</i> ^a ; <i>Fitzroya cupressoides</i> ^a ; <i>Actinostrobus acuminatus</i> ^a	1.536	MT/MT
<i>Callitris</i> , Cupressaceae, (Pye et al. 2003; PHYML)	<i>pauper</i> ^b	0.00197	<i>glauc</i> ^b	0.00976	<i>C. papuana</i> ^b ; <i>Allocasuarina zephyra</i> ^b	4.954	MT/MT
	<i>microphylla</i> ^c	0.00480	<i>glycinoides</i> ^b	0.02118	<i>C. tashiroi</i> ^a ; <i>C. gentianoides</i> ^a ; <i>C. afoliata</i> ^a	4.413	CL/CL
	<i>attenuatus</i> ^a	0.00810	<i>cotinifolius</i> ^b	0.01459	<i>Gyrostemon thesioides</i> ^a ; <i>Reseda valentina</i> ^a	1.801	MT/MT
<i>Daviesia</i> , Fabaceae: Faboideae, (PHYML)	<i>ulicifolia</i> sub. <i>aridicola</i> ^d	0.00660	<i>ulicifolia</i> sub. <i>ulicifolia</i> ^d	0.09450	<i>D. arthropoda</i> ^d ; <i>D. acicularis</i> ^d	14.318	SH/SH
<i>Dodonaea</i> , Sapindaceae, (PHYML)	<i>lobulata</i> ^b	0.01195	<i>megazuga</i> ^c	0.00704	<i>D. viscosa</i> ^a ; <i>D. madagascariensis</i> ^a	0.589	SH/SH
	<i>dumdaui</i> ^a	0.00317	<i>cornuta</i> ^a	0.00477	<i>E. woodwardii</i> ^a ; <i>E. stoatei</i> ^a ; <i>E. siderophloia</i> ^b ; <i>E. torquata</i> ^a	1.505	MT/LT
<i>Exocarpos</i> , Santalaceae, (PHYML)	<i>vicinia</i> ^{c,e}	0.00563	<i>glaucina</i> ^{a,c}	0.00676	<i>E. pellita</i> ^a ; <i>E. brassiana</i> ^a ; <i>E. urophylla</i> ^a	1.201	ST/ST
	<i>salmonophloia</i> ^a	0.00665	<i>diversicolor</i> ^a	0.00825	<i>E. michaeliana</i> ^a ; <i>E. hallii</i> ^a ; <i>E. paludicola</i> ^a	1.241	LT/LT
	<i>porosa</i> ^c	0.00997	<i>siderophloia</i> ^b	0.01204	<i>E. cornuta</i> ^a ; <i>E. leucophloia</i> ^a ; <i>E. vicina</i> ^d ; <i>E. deglupta</i> ^a	1.208	MT/LT
	<i>aphyllus</i> ^b	0.02600	<i>cupressiformes</i> ^b	0.02753	<i>Osyris alba</i> ^a ; <i>Santalum macgregorii</i> ^a	1.059	RP/RP

<i>Flindersia</i> , Rutaceae, (Scott et al. 2000)	<i>maculosa</i> ^b	0.03858	<i>australis</i> ^b	0.05118	<i>Acronychia oblongifolia</i> ^a ; <i>Zanthoxylum simulans</i> ^a	1.327	MT/MT
<i>Grevillea</i> , Proteaceae, (PHYML)	<i>striata</i> ^b	0.00651	<i>robusta</i> ^a	0.01034	<i>Hakea leucopetra</i> ^b ; <i>H. salicifolia</i> ^b ; <i>Opisthiolepis heterophylla</i> ^a ; <i>Lomatia silaifolia</i> ^a ; <i>Stenocarpus sinuatus</i> ^b	1.588	LT/LT
<i>Hakea</i> , Proteaceae, (PHYML)	<i>leucopetra</i> ^b	0.00694	<i>salicifolia</i> ^b	0.01158	<i>G. striata</i> ^b ; <i>G. robusta</i> ^b ; <i>Opisthiolepis heterophylla</i> ^b ; <i>Lomatia silaifolia</i> ^a ; <i>Stenocarpus sinuatus</i> ^b	1.669	SH/SH
<i>Hibiscus</i> , Malvaceae, (Pfeil et al. 2002)	<i>sturtii</i> ^b	0.12242	<i>heterophyllus</i> ^b	0.12146	<i>Gossypium exiguum</i> ^a ; <i>Abutilon grandifolium</i> ^a ; <i>Cristaria insularis</i> ^a	0.992	SH/SH
<i>Jasminum</i> , Oleaceae, (PHYML) <i>Leptospermum</i> ^c , Myrtaceae, (PHYML) <i>Melaleuca</i> , Myrtaceae, (Cook et al. 2008; PHYML)	<i>lineare</i> ^b	0.02338	<i>volubile</i> ^b	0.12896	<i>J. humile</i> ^a ; <i>J. nudiflorum</i> ^a	5.516	SH/SH
	<i>coriaceum</i> ^b	0.03763	<i>laevigatum</i> ^b	0.03896	<i>L. petersonii</i> ^b ; <i>K. ericoides</i> ^b	1.035	SH/SH
	<i>brachyandrus</i> ^c	0.01046	<i>saligna</i> ^b	0.01176	<i>M. leucadendra</i> ^a ; <i>M. argentea</i> ^a ; <i>M. glomerata</i> ^a ; <i>M. lanceolata</i> ^a ; <i>Metrosideros perforata</i> ^a	1.110	ST/ST
	<i>glomerata</i> ^a	0.02510	<i>quinquenergia</i> ^a	0.02787	<i>M. comboyensis</i> ^a ; <i>M. brachyandrus</i> ^d ; <i>Metrosideros perforata</i> ^a ; <i>Choricarpia subargentea</i> ^a	1.124	MT/MT
<i>Myoporum</i> , Scrophulariaceae, (PHYML)	<i>platycarpum</i> ^b	0.01861	<i>acuminatum</i> ^f	0.03242	<i>M. parvifolium</i> ^a ; <i>Eremophila rotundifolia</i> ^a ; <i>E. macdonnellii</i> ^a	1.742	ST/ST
<i>Pittosporum</i> , Pittosporaceae, (Chandler et al. 2007; PHYML)	<i>angustifolium</i> ^b	0.06366	<i>undulatum</i> ^b	0.03169	<i>P. crassifolium</i> ^a ; <i>P. tenuifolium</i> ^a ; <i>Billardiera heterophylla</i> ^a ; <i>Marianthus bicolor</i> ^a	0.498	MT/MT
<i>Santalum</i> , Santalaceae, (Harbaugh & Baldwin 2007)	<i>lanceolatum</i> ^a	0.01032	<i>obtusifolium</i> ^a	0.03302	<i>S. murrayanum</i> ^a ; <i>S. spicatum</i> ^a ; <i>S. acuminatum</i> ^a	3.200	RP/RP
<i>Senna</i> , Fabaceae: Caesalpinioidea	<i>phyllodinea</i> ^c	0.03289	<i>odorata</i> ^a	0.04288	<i>Caesalpinia sappan</i> ^a ; <i>Pomaria melanosticta</i> ^a ; <i>Zuccagnia punctata</i> ^a ; <i>Hoffmannseggia yariensis</i> ^a	1.304	SH/SH
<i>Templetonia</i> , Fabaceae: Faboideae, (Thompson et al. 2001; PHYML)	<i>egena</i> ^b	0.00312	<i>retusa</i> ^a	0.02617	<i>Hovea elliptica</i> ^a ; <i>H. trisperma</i> ^a	8.388	SH/SH

^aSequence sourced from GenBank.^bSequence sourced from fresh tissue collected by authors.^cSequence sourced from seedlings raised in laboratory.^dSequence sourced from Mike Crisp.^eIndicates comparison where one comparator recorded 0 rate from ingroup node; distance to next node included for both comparators.^fSequence sourced directly from seed.

a taxonomically diverse sample range. Each pairing contrasted a species from the arid zone with a congener from the mesic zone, rooted by two to six proximate (congeneric and confamilial) outgroup taxa, to ensure a robust proxy for the ancestral ITS sequence. Wherever possible, we selected taxa for comparison on the basis of previously published phylogenies (table 1 and electronic supplementary material). Independent phylogenetic contrasts have been established as a robust method for examining the potential effect of a broad range of variables on the evolutionary process (Harvey & Pagel 1991).

A number of factors other than productivity could potentially account for any rate differentials that might be evident between arid and mesic taxa, including generation time, cladogenetic signals, population size and attendant genetic drift, or temperature (Ohta 1992; Rohde 1992; Wright *et al.* 2006, 2009). In addition, gene flow between species pairs and alternative water sources to arid species might assuage any asymmetry in genetic evolution. Therefore, in order to reduce the potential confounding influence of such non-target processes on the molecular record, we applied rigorous *a priori* criteria to the selection of the comparator species. Consequently, the two contrasted species in each pairing were selected on the basis of: minimizing the phylogenetic distance between them; minimizing differences in latitudinal range; reducing the effect of population size by ensuring similar range sizes and avoiding rarities; ensuring similar life histories by requiring similar habits for each member of the pair; ensuring allopatry by maximizing the geographical distance between them and; avoiding arid taxa with access to alternative water supplies. The rationales for these criteria are expanded upon below.

Ensuring that the contrasted species are as closely related as possible increases the ability to detect differences in substitution rates that can reasonably be attributed to the target variable (Wright *et al.* 2009). Such a precaution minimized the potential influence of phylogenetic and demographic processes, including differential rates of cladogenesis, the molecular signal of which may otherwise accumulate with increasing phylogenetic distance (Pagel *et al.* 2006). Accordingly, wherever possible arid-mesic congeners were sister taxa or, where the first sister did not meet the other criteria of our study, the next most closely related species that did satisfy those criteria was chosen. Further, to control for the potentially confounding signal of the node density effect (Hugall & Lee 2007), where appropriate, we pruned other ingroup taxa prior to ML rate estimation. Constraining phylogenetic distance and pruning have been applied as precautionary steps in other comparative studies of this kind (Bromham & Cardillo 2003; Gillman *et al.* 2009; Wright *et al.* 2009). Smaller populations may support faster micro-evolutionary rates owing to a higher probability of fixation for mildly deleterious mutations (Ohta 1992). However, in contrast to this prediction of nearly neutral theory, the recent empirical finding that bird species with smaller populations have slower rates of DNA evolution was also relevant for the need to control for a population size effect (Wright *et al.* 2009). Therefore, we avoided species designated as rare or threatened, and preferentially selected arid-mesic contrasts involving species with similar range sizes.

We selected ingroup pair members that had minimal latitudinal differences in geographical range in order to reduce the potential effect of latitude-based temperature variation on molecular evolution (Wright *et al.* 2006) and that

were distinctly allopatric in order to reduce the likelihood of hybridization that could, owing to concomitant gene flow, have disrupted our ability to detect differential substitution rates. Moreover, ingroup taxa were selected that represented the largest differences in rainfall, to maximize the potential effect of the target variable. We also ensured that the arid-mesic comparator species were typically of similar size and habit (woody sub-shrubs, climbers, shrubs, small trees, medium trees, large trees) in order to minimize any possible effect of life-history variation on micro-evolutionary rates (Smith & Donoghue 2008).

(b) *Tissue collection and sequence acquisition*

The combined stringency of these criteria meant that most of the ingroup samples had to be collected and sequenced by us, with a minority of the total ingroup dataset being derived from the public domain (GenBank). Field tissue collection occurred between August 2007 and November 2008 in arid and coastal New South Wales. Fresh leaf tissue was collected and stored at 4°C or dried over silica gel immediately after collection and stored at room temperature. We obtained seeds for some species from the Royal Botanic Gardens, Sydney. Some of these were raised to produce seedlings, from which DNA was extracted. Sequences obtained from the different sources, including GenBank accession numbers, are provided in the electronic supplementary material. We extracted genomic material using the QIAGEN (Valencia, CA, USA) DNeasy Plant Mini Kit (QIAGEN 69104) before PCR. The ITS primers we predominantly used in this study were *CY1* (forward: TACCGATTGAA TGATCCGGTGAAG) and *CY3* (reverse: CGCCGTTAC TAGGGGAATCCTTGT), which are suitable for most angiosperm samples. Some sequences proved either intractable with, or unsuitable for, angiosperm primers (e.g. the gymnosperm *Callitris*: Cupressaceae). For these sequences we used a more permissive 3' primer (JK18S: TACACA CCGCCCGTCGCTCCTACC) that binds further downstream from the *CY1* site, in a more widely conserved region of the 18S sequence. Only complete ITS data of nuclear rRNA-encoding DNA (ITS1-5.8S-ITS2) was used for all ingroup comparisons. The PCR cycle featured an initial 2 min hold at 94°C followed by 10 touchdown cycles of 94°C for 30 s, 65°C for 30 s decreasing by 1° each cycle to 56°C and 72°C for 1 min. This was followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and a final hold at 72°C for 5 min. Low levels of amplification from some samples necessitated that the temperature of the annealing phase be decreased from 60°C to 50°C in the touchdown cycles, or that the number of cycles be increased from 40 to 45 or 50; in some cases both modifications were used. For the *Callitris* sequences, extension times during the touchdown and lower temperature cycling were increased from 1 to 3 min, to take into account the generally longer ITS sequences for gymnosperms. We confirmed PCR products and negative controls via electrophoresis on 1 per cent agarose gel. PCR products were purified using the magnetic-bead based AMPure kit (Agencourt 000130) containing magnetic beads that bind DNA sequences of more than 100 bp. PCR product concentrations were quantified using a nano-drop spectrophotometer (ND-1000). We used direct sequencing methods based on the ABI Prism Big Dye Terminator Cycle (ABI 4337454) kit, using the *CY1* or *CY3* primers. For some sequencing reactions, we used additional primers that sequenced from the 5.8S gene

(JK5.8F: GATACTTGGTGTGAATTGCAGA; JK5.8R: ATGGTTCACGGGATTCTGCAA) to improve the quality of the sequencing read (e.g. for *Callitris*). The forward and reverse strands of each sequence were assembled using *Contig Express* (Vector NTI Suite 9.0.0, Informax). To avoid the potentially confounding effect of pseudospacers, we used the criteria established by Feliner & Rossello (2007) for detecting and avoiding paralogous ITS sequences in all instances where sequences were generated by us.

(c) Phylogenetic rate estimation and statistical procedures

Multiple sequence alignments were performed using the alignment program CLUSTALX v. 2 (Thompson et al. 1997; Larkin et al. 2007). Where no previously published phylogenetic analyses were available, or where ITS sequences for additional congeneric taxa to those published in phylogenetic literature were available, we performed preliminary ML phylogenetic reconstructions at the generic and familial level with all available congeneric sequences, from our own collection efforts or from the public domain. Where no additional sequences than those already published in the literature were available, we used those studies. For these phylogenetic reconstructions, we used the GENEIOUS v. 4.5.3 (Biomatters) PHYML plugin (Guindon & Gascuel 2003) using a general time reversible (GTR) substitution model bootstrapped 1000 times, with transition–transversion ratios, proportion of invariable sites and gamma distribution estimated by the program, and topology and branch length and rate parameters optimized during the procedure. We then pruned non-target ingroup taxa from the alignments, and retained the most proximate outgroups for subsequent rate estimation. In some instances (*Casuarina*, *Codonocarpus* and *Senna*), no additional congeneric sequences were available other than those collected by us; we subsequently could not perform topology optimization using PHYML for these comparisons, and proceeded directly to ML branch length estimation. ML branch length estimates were performed for the target ingroups and outgroups using GENEIOUS v. 4.5.3 PAUP* v. 4.0b10 for Mac OS X plugin (Swofford 2003). We employed a GTR model using a heuristic search, empirical base frequencies, estimated gamma shape parameter (four categories) and estimated proportion of invariant sites. Starting trees were obtained by stepwise addition with randomized input order and 10 replications. In the instances where one of the branch lengths displayed no change in the phylogenetic reconstruction, we summed both of the lineages with the branch leading from their node to the ingroup–outgroup node; this is a more conservative estimate of rate differentials. The rate estimates were used to generate ratios (highest/lowest) and we assigned a negative sign to the resultant ratios when the arid species had the longer branch length, and a positive sign when the mesic species had the longer branch. We used highest over lowest ratios in order to provide symmetry around the null hypothesis of median = zero. We tested for statistical significance using the Wilcoxon sign-rank test and the binomial sign test.

3. RESULTS AND DISCUSSION

The majority (76%) of mesic plant species exhibited higher substitution rates than arid species (sign test: $n = 30$, $p = 0.005$) and the rate of nucleotide substitution in mesic zone taxa was typically much greater than that of

closely related arid zone congeners (median ratio = 1.32; Wilcoxon sign-rank test: $n = 30$, $W = 93$, $p = 0.003$). A number of factors other than water availability could potentially account for this rate differential, including cladogenetic signals, or population size and attendant genetic drift, or temperature (Ohta 1992; Rohde 1992; Wright et al. 2006, 2009). However, as discussed previously, in order to avoid the potential confounding influence of these and other effects, we applied rigorous *a priori* criteria to the selection of the comparator species.

The relationship described here between water availability and substitution rate heterogeneity for woody species is analogous to relationships between available energy and molecular rate variation that have also been found for woody plants (Wright et al. 2006), amphibians (Wright et al. in press) and mammals (Gillman et al. 2009). Taken together, these results provide support for the prediction of the evolutionary speed hypothesis that molecular rates should covary positively not only with thermal energy (Rohde 1992), but more specifically with productivity (Gillman & Wright 2007) across a water-energy gradient. The earlier recorded latitudinal differences in micro-evolution, and the longitudinal one revealed in this research, would appear to be distinct yet inter-related facets of the same essential biological relationship based on productivity. Over large latitudinal extents, the constraining component of water-energy dynamics—to which many global biodiversity patterns are linked (Hawkins et al. 2003)—is temperature. Across such latitudinal gradients, it would thus appear that biological activity is governed by extrinsic ambient energy, and its ability to potentiate fundamental metabolic growth and trophic processes. By extension, in areas of high energetic input, but where water is the limiting factor—such as Australia—the same consequences for biological activity and diversity should apply (Hawkins et al. 2003). Thus, in an additional dimension, the evolutionary speed concept is applicable and testable relative to the second major component of the water-energy balance (Gillman & Wright 2006, 2007; Wright et al. 2006). Given the potential importance of such a combined relationship to global species richness patterns, this finding therefore expands the potential explanatory scope of the evolutionary speed hypothesis.

Central to the evolutionary speed hypothesis is the directionality of the relationship between genetic change and speciation events, because it is predicated on the assumption that faster rates of micro-evolution lead to greater rates of speciation and diversification. Thus, mutation and substitution are seen as key factors limiting speciation (Evans et al. 2005). There is evidence for covariation between diversification rates and molecular evolutionary rates for both neutral and coding DNA (Barracough & Savolainen 2001; Davies et al. 2004a,b). By contrast, nearly neutral theory proposes that rapid molecular evolution may occur during speciation and cladogenetic events as a consequence of the demographic effects (smaller populations) associated with speciation (Pagel et al. 2006). However, these predictions have not been borne out by recent comparative studies (Wright et al. 2006; Gillman et al. 2009). For example, analyses of molecular rates of tropical versus temperate angiosperms showed higher molecular rates in tropical species,

even when the tropical clade contained the same, or fewer, species than the contrasted temperate clade (Wright *et al.* 2006). Nonetheless, and regardless of their possible impacts, these putative effects were controlled for in this study both by our minimization of phylogenetic distance (and thus of the potential for differential cladogenetic signatures) within the ingroup pairings, and through the selection of ingroup species that minimized differences in population sizes.

Alternatively, the reproductive isolation of new species may be regulated by intrinsic rates of micro-evolutionary change within diverging populations. Barraclough & Savolainen (2001) argue that variation in mutation rates may drive phyletic change and speciation rates in angiosperms, with faster rates speeding up both the rate at which populations diverge and the appearance of reproductive isolation. This would certainly be relevant if the genetic component of reproductive isolation involved relatively small changes to certain loci. Thus, under a scenario where energy flux contributes to micro-evolutionary rate, as predicted by the evolutionary speed hypothesis, climatic impositions on the ability of living systems to use energy for biological functions could affect the rate of speciation and, by extension, the generation of diversity via genetic pathways (Rohde 1992; Wright *et al.* 2006). Further investigation of the direction of causality between molecular rates and speciation rates would provide more insight into these fundamental patterns.

Our results, showing higher micro-evolutionary tempos for plants living on the mesic margins of Australia, indicate that water availability may play a key role in evolutionary processes by regulating evolutionary speed. It appears that in environments with more optimal water-energy conditions, and resultant greater productivity, faster metabolic and growth rates in plants may increase rates of genetic evolution. The corollary is that the depauperate abiotic conditions in the Eremaean Zone of Australia may lead to depressed rates of micro-evolution owing to a reduction in biologically available energy and a concomitant decline in biological activity. That is, the effect of persistent drought may enforce slower rates of substitution in the arid-adapted plant occupants of the Outback.

In other regions of the world where water availability limits species richness, we might then find that evolutionary speed is also implicated. However, rates of molecular evolution may more closely relate to annual metabolic activity (Gillman *et al.* 2009) as it is influenced by rainfall, rather than to gross annual rainfall *per se*. Thus, where the seasonality of rainfall produces an excess of water in a short wet season, followed by prolonged water shortages, we might expect slower rates of DNA evolution relative to the same rainfall that is spread over a longer growing season. Further empirical examination of these relationships would assist in identifying the nature and magnitude, if any, of these other effects.

Our results support a putative causal relationship between environmental water-energy regimes, energetic flux and micro-evolutionary heterogeneity beyond a dimension that is solely latitudinal (as was originally proposed by the evolutionary speed hypothesis). Rather, these data suggest that rate heterogeneities for genetic change may exist across any broad-scale geographical

gradient in the energy-water balance, and that evolutionary speed may act to generate and mediate species richness across these diverse spatial extents.

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