Identification and dynamics of a cryptic suture zone in tropical rainforest

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Suture zones, shared regions of secondary contact between long-isolated lineages, are natural laboratories for studying divergence and speciation. For tropical rainforest, the existence of suture zones and their significance for speciation has been controversial. Using comparative phylogeographic evidence, we locate a morphologically cryptic suture zone in the Australian Wet Tropics rainforest. Fourteen out of 18 contacts involve morphologically cryptic phylogeographic lineages, with mtDNA sequence divergences ranging from 2 to 15 per cent. Contact zones are significantly clustered in a suture zone located between two major Quaternary refugia. Within this area, there is a trend for secondary contacts to occur in regions with low environmental suitability relative to both adjacent refugia and, by inference, the parental lineages. The extent and form of reproductive isolation among interacting lineages varies across species, ranging from random admixture to speciation, in one case via reinforcement. Comparative phylogeographic studies, combined with environmental analysis at a fine-scale and across varying climates, can generate new insights into suture zone formation and to diversification processes in species-rich tropical rainforests. As arenas for evolutionary experimentation, suture zones merit special attention for conservation.

Keywords: Australia; Wet Tropics; secondary contact zone; hybrid zone; habitat suitability model; speciation

1. INTRODUCTION

The climate oscillations of the Quaternary had profound effects on the distributions of habitats and the organisms that depend on them (Jansson & Dynesius 2002). For many biotas, this resulted in contraction of populations to disjunct refugia during the glacial periods that dominated much of the past two million years with expansions during permissive interglacial periods such as the present (Hewitt 2004). As it has long been recognized, one potential outcome is the formation of suture zones, regions where whole assemblages of species establish secondary contact between long-isolated lineages, in some cases hybridizing and in others forming tight parapatric boundaries (Anderson 1949; Remington 1968; Swenson & Howard 2005). Such zones provide an opportunity to compare levels and patterns of reproductive isolation in relation to divergence history and phenotypic evolution of taxa (Remington 1968; Hewitt 1988).

Comparative phylogeography, along with increasing availability of fine-grain distribution data and geographic information systems (GIS) modelling, has led to the resurgence of interest in defining and studying suture zones (Hewitt 2000, 2004; Swenson & Howard 2004, 2005; Rissler *et al.* 2006; Swenson 2006). This is for two reasons: first, using comparative phylogeography, we can

detect morphologically cryptic evolutionary lineages, thereby expanding the number of sister-lineages that can be compared beyond those recognized as subspecies or species; second, given appropriate sampling (Moritz *et al.* 2000; Bridle *et al.* 2004), phylogeographic evidence can distinguish between secondary contacts of previously isolated lineages, versus the alternative hypotheses of parapatric differentiation, i.e. establishment of phenotypic or genetic clines within a continuous population across a sharp environmental gradient (Endler 1977). To distinguish these hypotheses, Endler (1982*a,b*; see also Coyne & Orr 2004) argued that under the secondary contact hypothesis, contact zones should be spatially clustered at the mid-point between refugia and divergence times between sister-lineages should be similar (Swenson & Howard 2005; Whinnett *et al.* 2005); whereas, for the parapatric divergence model, contact zones should consistently be located on the environmental gradients. A related prediction is that within such regions, contact zones formed by secondary contact should be located in, or move towards, regions of the lowest population density (Barton & Hewitt 1989). Alternatively, contact zones could be concentrated in regions where hybrids have higher fitness than either parent (e.g. Moore 1977). Further, as emphasized by Endler (1982*a,b*), concentration of contact zones in intermediate or low suitability habitats alone does not itself distinguish between parapatric and allopatric modes of divergence. To do this, we
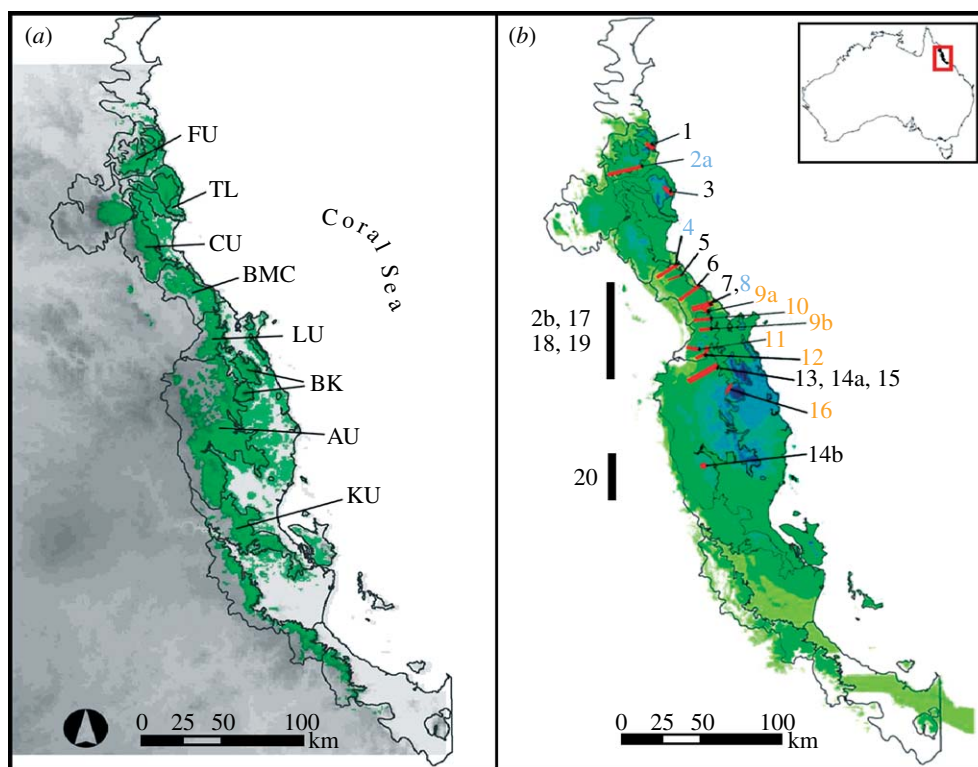
Figure 1. Australian Wet Tropics bioregion and location of contact zones relative to predicted refugia. (*a*) distribution of rainforest in the present climate (green). Topography is in grey; darker areas are higher elevations. Sub-regions: AU, Atherton Uplands; BK, Bellenden Ker/Bartle Frere; BMC, Black Mountain Corridor; CU, Carbine Uplands; FU, Finnigan Uplands; KU, Kirrama Uplands; LU, Lamb Uplands; TL, Thornton Lowlands; (*b*) spatial model of forest refugia (VanDerWal *et al.* 2008). Darker colours represent predicted higher stability since the Last Glacial Maxima. Red lines show contact zones, numbered as in table 1. Secondary contact is inferred from hybridization (taxa in orange), admixture for mtDNA (blue) and parapatry for mtDNA (black); numbers refer to table 1. Inset at the top right shows location of the Wet Tropics in the North-eastern Australia.

need to combine environmental analysis of contact zones with extrinsic evidence on biogeographic history, as can be obtained from palaeo-ecology, palaeo-modelling of habitats, phylogeography or some combination of these.

Considerable attention has been given to characterizing contact zones in the neotropical rainforest biotas because of debate over the existence and significance of mesic refugia during glacial periods of the Pleistocene (Haffer 1969; Endler 1982*b*; Haffer & Prance 2001). In particular, it has been questioned whether Amazonian contact zones are spatially clustered between putative refugia as predicted (Endler 1982*b*; Beven *et al.* 1984), and whether this rainforest even contracted during glacial periods (Colinvaux & De Oliveira 2001; Bush 2005; cf. Carnaval & Moritz 2008 for Atlantic Forests). Further complications are that for most sister-species pairs, divergence times predate the Pleistocene climatic cycles (Moritz *et al.* 2000; Rull 2008), and that divergence times can vary within a single suture zone (Whinnett *et al.* 2005). One intriguing study revealed geographically clustered phylogeographic breaks for small mammals in the western Amazon, but these related to an ancient geological ridge, rather than divergence among Pleistocene refugia (Patton *et al.* 2000). Overall, meta-analyses of contact zones in the highly diverse and complex Amazonian system are yet to resolve the long-running debates about the historical biogeography and speciation in the neotropics.

Here, we describe and analyse a suture zone in the Australian 'Wet Tropics' rainforest; a much simpler system, for which a history of refugial isolation during glacial periods and subsequent expansion and secondary contact during the Holocene is well established. These rainforests are small in extent (less than 1 Mha) and are distributed in a semi-continuous fashion along a north-south system of coastal ranges spanning some 400 km, and covering 0–1600 m in elevation (figure 1*a*). The distribution of the rainforest is largely controlled by climate, especially rainfall that is driven by orographic interception of moist, warm air from the adjacent Coral Sea. The climatic oscillations of the Late Quaternary, with greatly reduced temperature and rainfall at the Last Glacial Maximum (LGM, 18 thousand years before present, 18 Kya), followed by a permissive cool–wet phase in the Early Holocene (8–7.5 Kya) and then a warm-wet phase (5–3.5 Kya) prior to the establishment of current conditions (Kershaw & Nix 1988), had profound effects on the distribution of rainforests and of rainforest-dependent fauna in the Wet Tropics (Williams & Pearson 1997; Graham *et al.* 2006). Retraction of Wet Tropics rainforest to refugia during restrictive climates, the LGM in particular, is supported by evidence from palynology (Kershaw 1994; Kershaw *et al.* 2005), phytogeography (Webb & Tracey 1981) and also by spatial modelling of rainforests under varying climates (Nix 1991; Graham *et al.* 2006; Hilbert *et al.* 2007; VanDerWal *et al.* 2008). Predicted Late Quaternary refugia (figure 1*b*) vary in spatial scale and connectivity, with a major disjunction centred on the Black Mountain Corridor (BMC) that separates multiple small refugia in the north from a major refugial area along and adjacent to the escarpment of the

Table 1. Summary of contact zones mapped or inferred in this study. (D_A refers to net sequence divergence for mtDNA.)

species/lineage	contact zone location	evidence	D_A (mt gene)	evidence for lineage distinction	references
(a) Identified contact zones					
1. *Cophixalus aenigma/exiguus* FU	Mt Finnigan—Big Tableland, FU	mtDNA, morphology & call parapatry (1 km)	0.056 (12S/16S)	morphology, mating call	Hoskin (2004)
2. *Carphodactylus laevis* FU/N	(a) Mt Boolbun, FU	mtDNA admixture	0.033 (ND4)		Schneider *et al.* (1998) and Schneider & Moritz (1999)
3. *Saproscincus basiliscus/lewisi*	Cape Tribulation, TL	mtDNA parapatry (2 km), morphology	0.15 (ND4)	morphology	Moussalli *et al.* (2005, in press)
4. *Nyctimystes dayi* N/S	northern BMC	mtDNA parapatry (±10 km)	0.056 (CO1)	nuclear genes	M. Cunningham, J. MacKenzie & R. Bell 2008, unpublished data
5. *Glaphyromorphus fuscicaudis*	northern BMC	mtDNA parapatry (±10 km)	0.026 (ND4)		D. Truong, A. Carnaval, S. E. Williams & C. Moritz 2008, unpublished data
6. *Gnypetoscincus queenslandiae* N/S	Flaggy Ck, central BMC	mtDNA admixture	0.066 (ND4)	nuclear allozymes	Moritz *et al.* (1993), Schneider *et al.* (1998)
7. *Litoria rheocola* N/S	Streets Ck, southern BMC	mtDNA admixture	0.019 (CO1)	nuclear genes	Schneider *et al.* (1998) and J. MacKenzie, R. Bell & C. Hoskin 2008, unpublished data
8. *Saltuarius cornutus* N/S	southern BMC	mtDNA parapatry (±5 km)	0.087 (ND4)		Schneider *et al.* (1998) and Schneider & Moritz (1999)
9. *Litoria genimaculata* N/S	(a) Barron River, southern BMC; (b) Clohesy River, southern LU	mtDNA admixture, narrow hybrid zones, reinforcement	0.15(CO1)	nuclear genes, mating call	Hoskin *et al.* (2005) and J. MacKenzie, R. Bell & C. Hoskin 2008, unpublished data
10. *Temnoplectron reyi/politulum*	northern LU	mtDNA parapatry (±5 km), morphology	0.042 (CO1)	morphology	Bell *et al.* (2007)
11. *Bettongia tropica* N/S	Emu Ck, south LU	mtDNA admixture, hybridization	0.04 (control region)		Pope *et al.* (2000)
12. *Carlia rubrigularis* N/S	Lake Tinaroo, south LU	mtDNA, admixture, narrow hybrid zone	0.11 (cytb)	nuclear genes	Phillips *et al.* 2004*a* and Dolman & Moritz (2006)
13. *Lampropholis coggeri* N/C	Lake Barrine–Lake Eacham, northern AU	mtDNA parapatry (±2 km)	0.094 (ND4)	nuclear genes	R. Bell *et al.* (unpublished data)
14. *Gnarosophia bellendenkerensis* LU/AU; AU/KU	(a) Lake Barrine, northern AU; (b) Koombaloomba, southern AU	mtDNA admixture	0.06–0.15 (CO1)		Hugall *et al.* (2002)
15. *Austrochaperina robusta/fryi*	Lake Barrine, northern AU	mtDNA admixture, morphology, calls	0.09 (12S+16S)	mating call	Hoskin (2004)
16. *Cophixalus ornatus* N/S	Mt Bartle Frere, BK	mtDNA, nuclear admixture	0.06 (16S)	nuclear genes mating call	C. J. Hoskin *et al.* (unpublished data)
(b) Predicted contacts (spatial models predict continuity, but sampling inadequate to locate contacts)					
2b. *Carphodactylus laevis*	BMC	predicted	0.034 (cyt b)		Schneider *et al.* (1998)
17. *Eulamprus tigrinus*	BMC-LU	predicted	0.09 (ND4)		J. Mackenzie 2008, unpublished data
18. *Hypsilurus boydii*	BMC-LU	predicted	0.013 (ND4)		
19. *Litoria nannotis*	BMC	predicted	0.07 (COI)		Schneider *et al.* (1998)
20. *Saproscincus czechurai*	KU	predicted	0.10 (ND4)		Moussalli *et al.* (in press)

central Wet Tropics. The main features of this model are consistent with comparative phylogeographic evidence and stability surfaces from species-specific models (Hugall *et al.* 2002; Bell *et al.* 2007; Moussalli *et al.* in press), though the molecular data suggest considerable variation between species in the temporal and spatial scale of response (Schneider *et al.* 1998; table 1). Subsequent to the LGM, rainforests expanded rapidly across the upland areas, especially during the cool–wet phase of the Early Holocene (Hopkins *et al.* 1993). It was during this climatically favourable period that species ranges could have been maximally expanded from refugial areas, resulting in the formation of multiple zones of secondary contact. For higher elevation taxa, however, connectivity may have been temporarily disrupted during the warm phase of the Mid-Holocene (Winter 1997; Bell *et al.* 2007; Moussalli *et al.* in press).

Our aim here is to exploit this well-characterized system to identify and examine the characteristics of a suture zone in a tropical rainforest setting. We summarize published and new data on locations of secondary contacts between major phylogeographic lineages and (in a few cases) between sister-species. Using GIS, we evaluate the location of these secondary contacts relative to major refugia and focusing on the region between the major northern and central refugia, test whether the contact zones are spatially clustered and occur in regions of low environmental suitability (Barton & Hewitt 1989). Finally, we review current evidence regarding interactions between sister-lineages within the suture zone in relation to the extent of reproductive isolation versus divergence time (Coyne & Orr 2004).

2. MATERIAL AND METHODS

(a) *Locating contact zones*

We used mtDNA phylogeographies to locate parapatric boundaries between sister-lineages for 20 taxa, mostly amphibians and reptiles (table 1). In several cases, there is supporting evidence from nuclear gene sequencing for the assumption that major mtDNA phylogroups correspond to independently evolving lineages. As morphology is uninformative in most cases (see below), successive rounds of field sampling and mtDNA sequencing of populations were required to locate boundaries between major phylogeographic lineages. In each case, there is evidence for either (i) hybridization (from analysis of nuclear markers, or in one case from detection of intermediate calls), (ii) mtDNA admixture (but no corresponding analyses of nuclear markers to test for hybridization) or (iii) close (less than 20 km) parapatry of lineages. For several other taxa (table 1), species distribution models (Williams 2006) predict range continuity but sampling is too sparse to locate boundaries except on a very coarse scale. We exclude (mostly montane) phylogeographically structured species or species-pairs for which, based on point locality records and distributional modelling, there is unlikely to be current connectivity between lineages (e.g. montane *Cophixalus* (Hoskin 2004), *Pseudocheirus herbertensis/cinerea*, *Lampropholis robertsi*, *Saproscincus czechurai* and southern versus central lineages of *Saproscincus basiliscus* (Moussalli *et al.* in press). To represent known contact zones in GIS, contacts were described as points (for isolated records of admixture) or as lines and buffered by 2 km on both size (4 km total), unless fine-scale mapping

indicated narrower clines. The latter situation applies to *Carlia rubrigularis* (Phillips *et al.* 2004a), *Litoria genimaculata* (Hoskin *et al.* 2005), *Cophixalus ornatus* (C. J. Hoskin, M. Tonione, J. MacKenzie & C. Moritz 2008, unpublished data) and *S. basiliscus/lewisi* (Moussalli *et al.* in press), each of which was represented as a line with a 1 km buffer.

(b) *Spatial and environmental analysis of contact zones*

To examine clustering of contact zones along the dominant north–south axis, we treated the study area as one-dimensional, as seems reasonable given the region's geometry. To assess significance of observed spatial clustering, we applied randomization tests to the sum of all pairwise distances between contact zones (along the 430 km one-dimensional axis for the rainforest region, and a specified transect for the suture zone); a low score for this sum indicates clustering, while a high score would indicate overdispersion. To generate a null distribution, we placed random points ($n=18$ for the whole region, $n=14$ for the suture zone) along the axis, summed the pairwise distances for this random set and then iterated this process 9999 times. Since we expected clustering, we treated this test as one-tailed, testing for a sum of pairwise distances less than that observed. Similarly, we used randomizations to test whether contacts within the suture zone are centrally located or biased towards the northern or southern refugia; here two-tailed tests were applied to the test statistic—the sum of differences between zone location and the mid-point of the transect.

For the area between the major northern and central Wet Tropics refugia (figure 1*b*), we used two approaches to examine the location of contacts relative to environmental gradients. First, we used principal component analysis (PCA) based on bioclimatic variables extracted using randomly dispersed points from each of the refugia (Carbine Uplands, CU; $n=499$ points) and Atherton Uplands and Bellenden Kerr Range (AU/BK, $n=3205$ points) and the contact zones within the suture zone ($n=227$ points). Seven bioclimatic variables, selected on the basis of relevance to the organisms in question and low inter-correlation, were used; mean annual temperature, mean diurnal range of temperature, maximum temperature of warmest period, minimum temperature of coldest period, annual precipitation, precipitation seasonality (i.e. standard deviation) and the driest quarter moisture index.

Second, we used distributional modelling to see whether contact zones are located in regions of currently low environmental suitability for one or both of the lineages expanding from the adjacent refugia. We used Maximum entropy modelling (Maxent; Phillips *et al.* 2004b, 2006) to map spatial patterns of environmental suitability for rainforest (and adjacent wet sclerophyll forest as several species extend into this habitat; Williams 2006) from each of the CU and AU/BK regions. Since climate differs between these two regions, we created one prediction based on points ($n=1493$) sampled from CU and a second prediction based on 9410 points sampled from the larger AU/BK area. The number of random points extracted reflects the area of each sub-region and preliminary analyses indicated that this number adequately captures the environmental variation within each region. Maxent models were developed for both the current climate and the warm–wet period of the Mid-Holocene as described in VanDerWal *et al.* (2008). We then placed a transect through rainforest connecting the two refugia and

passing through the centre of BMC and Lamb Upland (LU) regions. Along this transect, we determined the environmental suitability (based on the predicted value from the Maxent model of each refugium) at 1 km steps. Analogous to the previous spatial analyses, we used randomizations to test whether contact zones are: (i) spatially clustered, (ii) located closer to one refugium than the other or located in regions of (iii) low environmental suitability (summed for AU/BK and CU) or (iv) similarly low environmental suitability relative to adjacent refugia (as predicted by the idea that secondary contacts should move to density troughs, using the sum of squared differences between AU/BK and CU values). Since we had clear expectations as to the direction of expected effects in cases i, iii and iv, one-tailed tests were employed in these tests, whereas a two-tailed test was used to test for unequal proximity to refugia (ii).

3. RESULTS

The analysis is unusual, though not unique (Hewitt 1988, 1999), among suture zone studies in having a high representation of morphologically cryptic lineages. In total, we mapped 18 contact zones for 16 taxa, and a further five contacts are predicted based on continuity of distribution models (table 1; figure 1*b*). These included two separate contacts between the northern and southern lineages of *L. genimaculata*, and two involving three lineages of *Gnarosophia bellendenkerensis*. Of the 18 mapped contacts, 14 involve morphologically cryptic lineages revealed through mtDNA phylogeography; the other four concern contacts between phenotypically diagnosable sister-species (*Cophixalus aenigma/exiguus*, *S. basiliscus/lewisi*, *Temnoplectron reyi/politulum*) or congeners (*Austrochaperina robusta/fryi*). Only the deepest phylogeographic divisions within species were considered, with levels of net mtDNA sequence divergence varying from 2 to 15 per cent. For all but a few comparisons, congruent patterns of nuclear gene or morphological variation support the assumption that mtDNA phylogeographic breaks correspond to boundaries between long isolated lineages (table 1).

Overall, contact zones were spread across the northern and central Wet Tropics, but were strongly clustered ($p < 0.001$). Most notably, 14 out of the 18 mapped contact zones occur in the relatively small and linear rainforest corridor between the major northern (CU) and central (BK/AU) refugial areas, and this pattern is strengthened (18/23), if we include other zones not yet mapped, but expected to occur based on predicted continuity of distributions between phylogeographic units (table 1; figure 1*b*). This effectively defines a 'suture zone', extending from the southern limit of CU, across the rainforests of the BMB, LU and northeastern AU (referred to as the Gadgarra break by Winter 1997) to the northwestern flanks of Mount Bartle Frere (BK). Within this suture zone, small high areas of the LU region are predicted to have been relatively stable, albeit highly fragmented, during the LGM (Hopkins *et al.* 1993; figure 1*b*). We expect that only species with very small area requirements were able to persist in the LU refuge and this is supported by the presence of distinct phylogeographic lineages centred on LU for the snail *G. bellendenkerensis* (Hugall *et al.* 2002), the lizard *L. coggeri* (R. Bell, J. MacKenzie & C. Moritz 2008,

unpublished data), and perhaps the microhylid frog *C. ornatus* (C. J. Hoskin, M. Tonione, J. MacKenzie & C. Moritz 2008, unpublished data), each of which has a contact zone between LU and AU/BK. Otherwise, the phylogeographies of individual species are more consistent with expansion into the suture zone from the larger CU and AU/BK refugia (e.g. Schneider & Moritz 1999).

The GIS environmental analyses provided insights into the locations of contacts within the suture zone. Multivariate analysis of environmental variables revealed clear separation between the CU and AU/BK refugial areas (figure 2). A PCA of climatic variables explained 44 per cent of total variation on axis 1 (temperature variables) and 34 per cent on axis 2 (precipitation variables). Relative to AU/BK, CU has higher seasonality of precipitation and a lower dry season moisture index. The contact zones mostly occupy intermediate environmental space especially on the precipitation axis, and many are located in warmer (i.e. lower elevation) areas of climate space relative to refugial areas (bottom right, figure 2).

Spatial analysis of environmental suitability through the suture zone, relative to each of the CU and AU/BK subregions, revealed marked asymmetry (figures 3*a* and 4). Relative to CU, there is moderate (although declining) suitability of habitats from north to south within the BMC and LU, whereas, relative to AU/BK there is a much more abrupt decline in suitability north of the LU, corresponding to a rain-shadow west of the BK mountains (i.e. the 'Gadgarra Rim'; Winter 1997). To the extent that species or phylogeographic lineages have evolved physiological differences between CU and AU/BK (see below), this would imply greater potential for migration from north to south than the opposite within the BMC and LU regions. For the warm–wet conditions estimated for the Mid-Holocene (Kershaw & Nix 1988), Maxent models predict unsuitable conditions relative to both adjacent refugia (except for high points in LU; figure 3*b*), suggesting that the current suture zone was established by recolonization subsequent to that period, i.e. within the past 3000 years.

Within the suture zone, there is some evidence that the contact zones are spatially clustered ($p = 0.072$ or $p = 0.0096$, where the latter is calculated excluding imprecisely located contacts 4 and 5; figure 4). Six out of the 14 contacts in this area are located to the north of the transect mid-point; such that there is no statistical trend for concentration of zones towards one refuge or the other ($p > 0.18$). Overall, there is a trend for contact zones to be located in areas of lower (summed) environmental suitability relative to adjacent refugia ($p = 0.046$ or $p = 0.082$ excluding contacts 4 and 5; figure 4). This is particularly evident relative to the AU/BK refuge, for which all but one zone is located in areas with less than 50 per cent suitability, and even the exception (*C. ornatus*) occurs in a localized area of lower suitability (figure 4). Furthermore, there is evidence that contacts are located in areas of equally low suitability, as predicted by the idea that secondary contacts will move to density troughs ($p = 0.027$ or $p = 0.036$ excluding contacts 4 and 5). The role of the Barron River as a barrier to gene flow, and hence as an attractant of contact zones, also bears consideration. Of the zones mapped, three (*S. cornutus*, *Litoria rheocola* and one of the two *L. genimaculata* contacts) are in close proximity (less
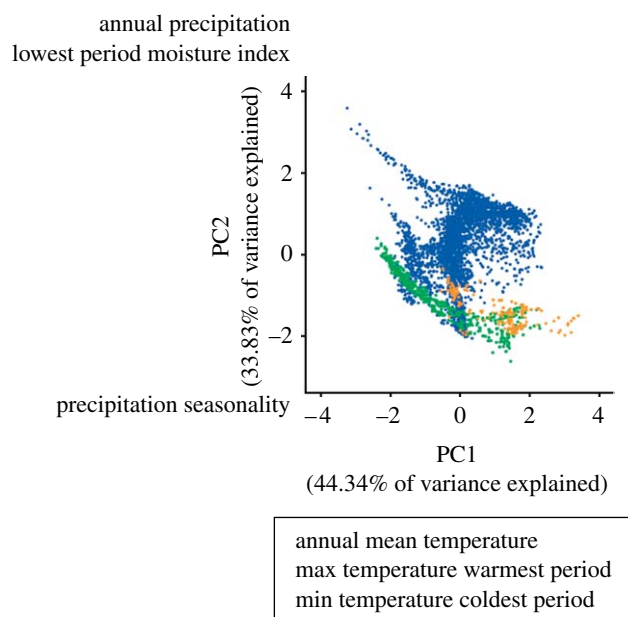
Figure 2. Principal Component Analysis based on environmental parameters sampled for refugia (CU, green; AU/BK blue) and secondary contacts within the suture zone (orange). Climatic variables contributing to each axis are shown. All variables in the box below the *x*-axis increase in value left to right. This analysis excludes the *C. ornatus* contact zone as that occurs largely within the BK refuge.

than 1 km), suggesting that the river could have acted as a local barrier to expansion for some taxa.

4. DISCUSSION

Suture zones have long been regarded as significant biogeographic features and as fascinating natural laboratories for studying divergence and speciation. The combination of comparative phylogeography and GIS-enabled environmental analysis holds great promise for enriching our understanding of such areas (Kozak *et al.* 2008; Swenson 2008). Such analyses have questioned some putative examples of suture zones (Beven *et al.* 1984; Swenson & Howard 2004), and others have identified climatic variables that define hybrid zones and differentiate the parental taxa (Kohlmann *et al.* 1988; Cicero 2004; Swenson 2006; Ruegg 2008). Proposed suture zones in tropical rainforests were central to hypotheses of allopatric divergence among Quaternary refugia, but the evidence from neotropical systems has been criticized from several perspectives (Endler 1982*a*,*b*; Beven *et al.* 1984; Whinnett *et al.* 2005). In the Wet Tropics, rainforests of northern Australia, the BMC/LU region offers a clear example of a suture zone, and one for which long-term isolation of rainforest-restricted fauna between adjacent refugia is well substantiated. In addition to the evidence presented here, the BMC/LU region has also been noted as a biogeographic barrier for mammals (Winter 1997) and is prominent, though not exclusively so, in biogeographic patterns of speciation in insects (Bouchard *et al.* 2005). An unusual feature of this suture zone is that most of the interacting lineages are morphologically cryptic, despite long histories of isolation, and it may be that comparative phylogeographic analyses of other tropical biotas will reveal other such morphologically cryptic suture zones.

It is commonly proposed that the lineages meeting within suture zones formed following allopatric divergence in adjacent refugia should have homogeneous divergence times (Endler 1982*b*; Coyne & Orr 2004; Whinnett *et al.* 2005). However, given repeated episodes of contraction and expansion during the Quaternary (Hewitt 2000), it might be expected that the most recent (Holocene) expansion might involve pairs of lineages that had initially diverged during different glacial periods, or perhaps even prior to the Quaternary (Hewitt 1999). In the present case, the lineage-pairs now in secondary contact within the BMC/LU suture zone exhibit a wide range of net-sequence divergence values (see also Hewitt 1999; Whinnett *et al.* 2005) and represent the outcomes of multiple vicariance events associated with climatic cycling during the Quaternary or even earlier (Schneider *et al.* 1998; Hugall *et al.* 2002). This does not deny that the present suture zone represents a common history of Holocene expansion from adjacent refugia. We suggest, therefore, that rather than simultaneous divergence times, a more rigorous criterion for defining suture zones is that the interacting lineages have a common time of *expansion*, rather than divergence. For the present case, the most likely period of expansion was the cold–wet period of the Early Holocene (ca 8–7.5 Kya), but it is also possible that cool-adapted lineages were again separated during the Mid-Holocene (5–3.5 Kya) warm–wet period (figure 3*b*) and expanded again in the Late Holocene. To discriminate among these alternatives would require coalescent analyses based on large samples sizes and multiple nuclear loci that are beyond the scope of the present analysis.

A key finding from the environmental analysis of the Wet Tropics suture zone is that the contact zones are concentrated in regions with low suitability relative to both adjacent refugia. This could simply reflect areas of first contact between rainforest (and hence lineages) following re-expansion from refugia, i.e. rainforest is likely to spread rapidly through areas of high suitability and then slow in areas of low suitability. However, our observations are also in accord with the prediction that hybrid zones should be located in density troughs (Barton & Hewitt 1989), or as a surrogate, areas of equally low environmental suitability for both lineages. In this case, the current position of contact zones does not necessarily represent the point of first contact between lineages; they may have subsequently shifted to areas of equally low environmental suitability (Kohlmann *et al.* 1988). To some extent, our analyses assume that long-term isolation within the environmentally distinct CU and AU/BK refugia has led to physiological divergence among phylogeographic lineages. This premise is supported for both cases, where thermal physiology has been compared between CU and AU populations (*C. rubrigularis* and *G. queenslandiae*; G. Langham, A. Krockenberger, S. E. Williams & C. Moritz 2008, unpublished data). That the contact zones are concentrated in the region of the lowest environmental suitability points to the need to assess ecological cost of movement, rather than distance *per se*, when assessing the location of suture zones relative to putative refugia. In the present case, the environmental asymmetry suggests that populations should be more able to expand southward from CU than northward from AU/BK.
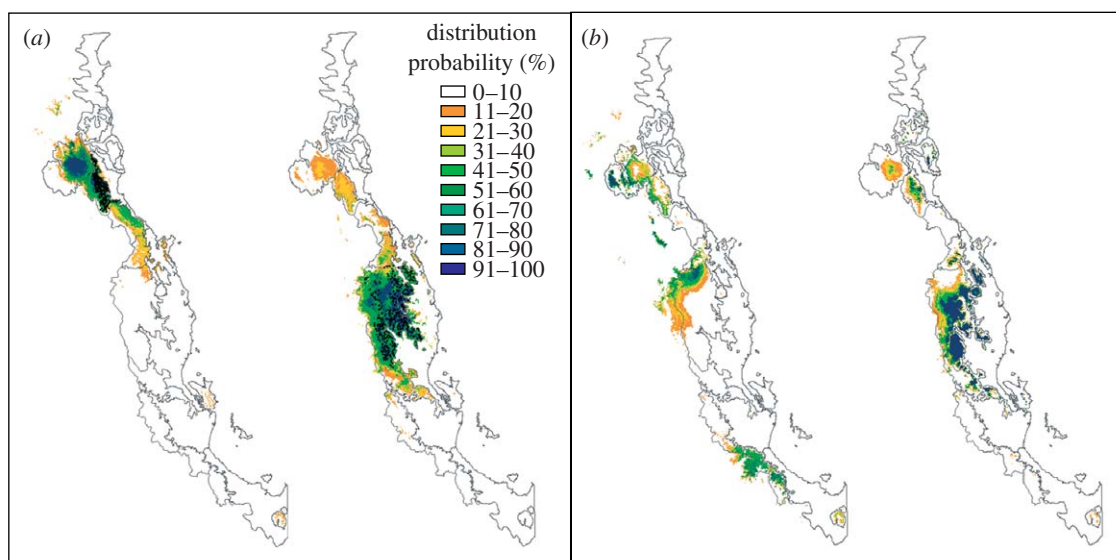
Figure 3. Maxent models for CU and AU/BK refugial areas (based on pre-clearing distribution of rainforest and wet sclerophyll forest) in (*a*) the current environment, and (*b*) the warm–wet climate of the Mid-Holocene. Black dots indicate the points from which the Maxent model was constructed.
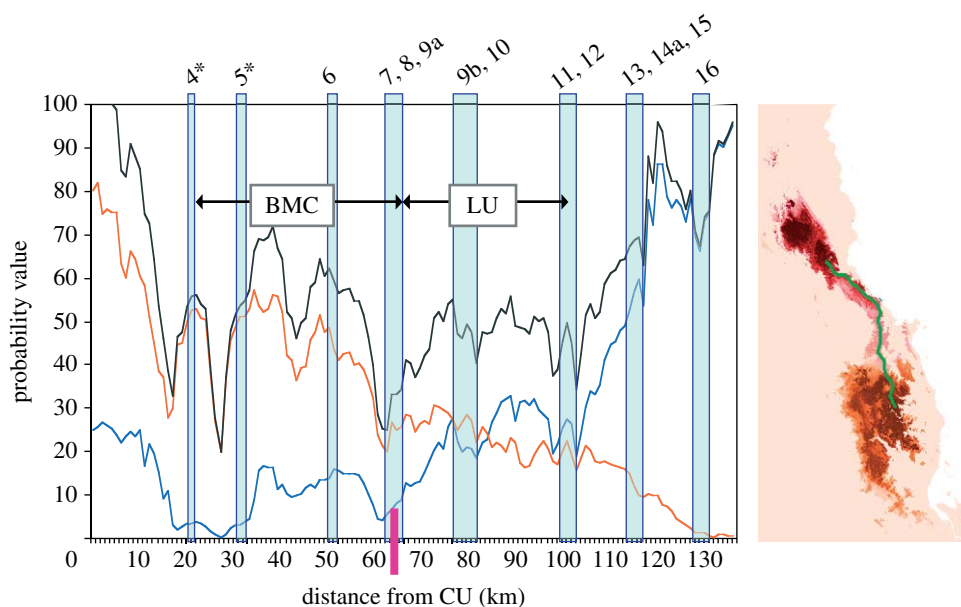

Figure 4. Fine-scale analysis of locations of contacts within the suture zone in relation to environmental gradients southward from CU (orange line), northward from AU/BK (blue line) and summed environmental suitability (dark grey line). The bars represent the locations of the contact zones (numbered according to table 1). Probability value refers to the Maxent models for CU and AU/BK (figure 3*a*), sampled via the transect (green line, inset to the right). The pink bar on the *x*-axis represents the location of the Barron River.

There has been a recent resurgence of interest in evolutionary phenomena in hybrid zones, ranging from selective filtering of adaptive mutations (Mallett 2005) through to hybrid-speciation (Mallett 2007) or the role of reinforcement in completing the speciation process (Servedio & Noor 2003). In general, it is expected that post-zygotic isolation increases with divergence time and that prezygotic isolation is stronger in sympatric than allopatric lineages (Coyne & Orr 2004). Suture zones offer an opportunity to compare the form and strength of reproductive isolation among interacting lineages in a common environmental setting (Remington 1968; Hewitt 1988). Only a small number of the contacts within the Wet Tropics suture zone have been studied in detail, but even so there is evidence for a range of outcomes. For the macropod, *Bettongia tropica*, there is no evidence of

genetic disequilibrium or reproductive isolation following secondary contact between lineages with relatively low genetic divergence (Pope *et al.* 2000). For the skink *C. rubrigularis*, in which lineages of intermediate divergence are in secondary contact, a multi-locus analysis revealed strong disequilibrium and substantial post-zygotic isolation but no assortative mating (Phillips *et al.* 2004*a*; Dolman 2008) and there is some evidence for introgression (Dolman & Moritz 2006). For the frog, *L. genimaculata*, there are two contact zones between highly divergent lineages, and experimental and molecular analyses revealed strong, asymmetric post-zygotic isolation and, in one of the zones, reinforcement-driven phenotypic divergence and pre-zygotic isolation resulting in rapid allopatric speciation (Hoskin *et al.* 2005; Hoskin 2007). Finally, diagnosable and evidently reproductively

isolated sibling species of dung beetles (*Temnoplectron reyi/politulum*) are parapatric in this region (Bell *et al.* 2007), and it is possible that other insect species have initiated or completed speciation within the suture zone (Bouchard *et al.* 2005). Given these initial data, and the presence of numerous contact zones between lineages of varying ages, the Wet Tropics suture zone presents a strong system for further exploring the varied evolutionary outcomes that can result from interaction between genetically differentiated lineages.

Finally, we note that such suture zones could well be overlooked when developing regional strategies for conservation. Relative to most other sub-regions in the Wet Tropics, the BMC and LU have few narrowly endemic species and accordingly rank somewhat low when applying conservation planning algorithms that seek to maximize representation of species in the fewest areas (Moritz *et al.* 2001). However, from the perspective of protecting evolutionary process, these relatively poorly protected regions warrant increased attention (Moritz 2002). The recent discovery of a new species of frog, *Litoria myola*, generated via localized reinforcing selection in the *L. genimaculata* contact zone and which is narrowly restricted to a region of the southern BMC under strong pressure for development, is a case in point (Hoskin 2007).

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