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Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest

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ABSTRACT

Aim We aim to propose validated, spatially explicit hypotheses for the late Quaternary distribution of the Brazilian Atlantic forest, and thereby provide a framework for integrating analyses of species and genetic diversity in the region.

Location The Atlantic forest, stretching along the Brazilian coast.

Methods We model the spatial range of the forest under three climatic scenarios (current climate, 6000 and 21,000 years ago) with BIOCLIM and MAXENT. Historically stable areas or refugia are identified as the set of grid cells for which forest presence is inferred in all models and time projections. To validate inferred refugia, we test whether our models are matched by the current distribution of the forest and by fossil pollen data. We then investigate whether the location of inferred forest refugia is consistent with current patterns of species endemism and existing phylogeographical data.

Results Forest models agree with pollen records and predict a large area of historical forest stability in the central corridor (Bahia), as well as a smaller refuge (Pernambuco) along the Brazilian coast, matching current centres of endemism in multiple taxa and mtDNA diversity patterns in a subset of the species examined. Less historical stability is predicted in coastal areas south of the Doce river, which agrees with most phylogeographical studies in that region. Yet some widely distributed taxa show high endemism in the southern Atlantic forest. This may be due to limitations of the modelling approach, differences in ecology and dispersal capability, historical processes not contemplated by the current study or inadequacy of the available test data sets.

Main conclusions Palaeoclimatic models predict the presence of historical forest refugia in the Atlantic rain forest and suggest spatial variation in persistence of forests through the Pleistocene, predicting patterns of biodiversity in several local taxa. The results point to the need for further studies to document genetic and species endemism in the relatively poorly known and highly impacted areas of Atlantic rain forests of north-eastern Brazil.

Keywords

Atlantic forest, biogeography, Brazil, forest refugia, Holocene, palaeoclimate modelling, palynology, phylogeography, Pleistocene.

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INTRODUCTION

The large number of known studies about the effects of late Pleistocene habitat fluctuations on patterns of diversity and endemism reflects a long-lasting debate about the existence and effects of Quaternary rain forest refugia (Haffer, 1969;

Prance, 1982; Brown, 1987; Colinvaux *et al.*, 2000; Urrego *et al.*, 2005; Bush & de Oliveira, 2006; Graham *et al.*, 2006). Based on extensive pollen data, palaeovegetation and palaeoclimatic studies, the clear picture emerging from equatorial forests such as Amazonia is that of historical persistence of forests in the central and western portions of the biome, with

fluctuation and replacement by semi-deciduous dry forest or savanna happening only near ecotonal boundaries (Mayle *et al.*, 2004; Bush *et al.*, 2007). Molecular data from Amazonian taxa reinforce this view (Lessa *et al.*, 2003), opposing Haffer's (1969) suggestion that the existence of multiple isolated Pleistocene forest refugia drove much of the diversification of the local fauna. In contrast to the Amazonian scenario, regional variation in palaeoclimates and evidence from palaeoecology, modelling and phylogeography suggest the occurrence of Pleistocene forest refugia in other, more subtropical, areas (Barrable *et al.*, 2002; Hugall *et al.*, 2002; Hughes *et al.*, 2005; Graham *et al.*, 2006).

In the absence of extensive palaeontological data, spatial modelling of species or habitats under current and palaeoclimate regimes can generate hypotheses about the potential existence and extent of stable (refugial) areas, against which observations on species and genetic diversity, or from the fossil record, may be compared (Kohfeld & Harrison, 2000; Alfano *et al.*, 2003; Cheddadi *et al.*, 2006). When applied to one diverse but spatially restricted tropical rain forest system (the Australian Wet Tropics), palaeoclimatic modelling of forests and forest-dependent species predicted contraction to montane and mesic refugia at the Last Glacial Maximum and improved prediction of narrow species endemism and phylogeography in taxa with limited dispersal (Hugall *et al.*, 2002; Graham *et al.*, 2006). To evaluate the hypothesis that palaeomodelling can generate predictions about stable versus unstable areas in other biologically diverse regions with similarly complex topography and steep environmental gradients, we here apply this approach to the rain forests of coastal Brazil. The broad aim is to provide a framework for understanding and predicting spatial patterns of genetic and species diversity.

Analogous to the Australian Wet Tropics, the Brazilian Atlantic rain forest is characterized by strong seasonality, sharp environmental gradients due to complex topography and orographic rainfall driven by the easterly winds from the tropical Atlantic (Fundação Instituto Brasileiro de Geografia e Estatística, 1993). This exceptionally diverse biome encompasses multiple vegetation types, including open, mixed and closed evergreen, semi-deciduous and deciduous forests (Fundação Instituto Brasileiro de Geografia e Estatística, 1988).

We modelled the spatial range of the Brazilian Atlantic forest for three climatic scenarios: current climate, a Holocene wet phase and a late Pleistocene colder and drier period. Our goals were two-fold. First, we aimed to test whether our climate-based models were matched by the current forest distribution and by available fossil pollen data. Because ecoclimatically stable areas are expected to retain high levels of endemism (Fjeldså & Lovett, 1997; Fjeldså *et al.*, 1999; Graham *et al.*, 2006), we then investigated whether the regions predicted to have remained stable across climatic fluctuations (i.e. inferred refugia for forest species based on the climatic models) were consistent with current patterns of species endemism in coastal Brazil, as well as with molecular phylogeographical data previously generated for

Atlantic forest taxa. The poorly known history of this biodiversity hotspot and its current level of threat (Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais, 1993) make this exercise desirable and timely from a conservation biology standpoint.

METHODS

Climatic modelling procedure

We modelled the spatial range of the Brazilian Atlantic forest defined both broadly and narrowly. We employed Brazil's official vegetation map with estimates of the original distribution (prior to human-driven clearance) of all unique local forest vegetation types (Fundação Instituto Brasileiro de Geografia e Estatística, 1988) to generate two point data sets. A data set representing the Atlantic forest biome in its official (broad) definition was generated from 1000 presence points randomly sampled from the entire distribution of the biome, including points in areas of deciduous, semi-deciduous, open, mixed and closed evergreen forests. A second data set, corresponding to a narrower definition, comprised 1000 presence points randomly sampled from areas of closed and open evergreen forest only.

Two modelling methods were used to generate predicted distribution maps of the forest at a 30'' spatial resolution: the ecological niche-envelope model implemented by BIOCLIM in DIVA-GIS (Hijmans *et al.*, 2005b), and the machine-learning maximum entropy model MAXENT (Phillips *et al.*, 2006). Despite its conceptual simplicity, BIOCLIM has proved effective for studies of biodiversity patterns worldwide (Hugall *et al.*, 2002; Hijmans *et al.*, 2005b; Graham *et al.*, 2006). Nonetheless, MAXENT has been shown to outperform BIOCLIM and other modelling methods when generating predictions of the current range of a given species (Elith *et al.*, 2006). The relative performance of these and other modelling methods, when projected into past or future climates, is poorly known to date (see Araújo *et al.*, 2005, for an example). Because model outputs often vary widely when extrapolated to other climate conditions (Thuiller, 2004; Araújo *et al.*, 2005), it is best to integrate results across multiple methods (Araújo & New, 2007).

Model projections were carried out based on seven climatic variables: annual mean temperature, temperature seasonality, mean temperature of the warmest and coldest quarters, annual precipitation and precipitation of the driest and wettest quarters (available at <http://www.diva-gis.org/climate.htm>, last accessed 11 January 2008). Climatic data were obtained from spatially explicit bioclimatic layers downloaded from the WORLDCLIM 1.4 data base (Hijmans *et al.*, 2005a) and cropped to span from latitude 12°47' N to 34°46' S and longitude 78°31' W to 35° W (Fig. 1). This spatial coverage was deliberately made larger than that occupied by the Atlantic rain forest to ensure that the training points used to generate the models under the current environment encompassed the full range of palaeoclimatic values.

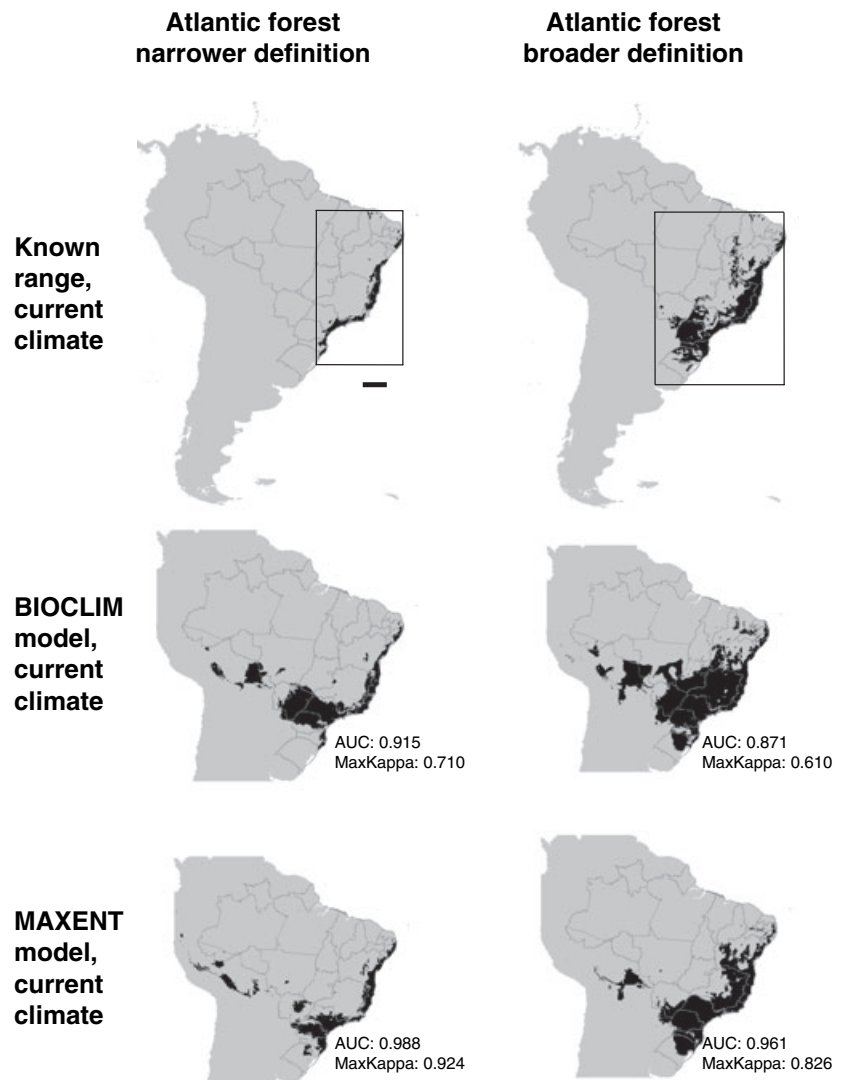


Figure 1 Top: known, pre-clearing ranges of the Atlantic forest under narrower and broader definitions. Mask areas used to extract points for the purposes of model testing are indicated. Middle: modelled ranges using BIOCLIM under current climate. Bottom: model ranges using MAXENT under current climate. Scale bar = 500 km.

We trained BIOCLIM and MAXENT models under current climatic conditions with a set of 750 presence points randomly sampled from the original 1000-locality data set. To test each model, we used a data file comprising the remaining 250 presence points plus 250 absence points randomly sampled from a pre-defined area. To test models of the distribution of the forest under a broader definition, we randomly extracted 250 absence points from natural (not human-driven) non-forest regions depicted in Brazil's vegetation map, using a mask extending from latitude 3° S to 34° S, longitude 58° W to 35° W. To test models of the forest under a narrower definition, we sampled 250 absence points within latitude 3° S to 30° S, longitude 50° W to 35° W (Fig. 1). Under both forest definitions, accuracy was checked given what is known about their true distributions as per Brazil's vegetation map. The use of the map was preferred over the modern pollen record given that the latter would provide fewer validating points and potentially add unnecessary biases or errors (Behling *et al.*, 1997).

We used maximum Kappa (Cohen, 1960) to establish a threshold and transform the continuous outputs of BIOCLIM

and MAXENT under current climatic conditions into presence/absence maps. We also used the area under the receiver operating characteristic curve (AUC; Hanley & McNeil, 1982) as a threshold-independent method for model comparison. After ensuring that model predictions under current climate were satisfactory, we used palaeoclimatic surfaces generated with the Paleoclimatic Modelling Intercomparison Project ECHAM3 atmospheric general circulation model (Deutsches Klimarechenzentrum Modellbetreuungsgruppe, 1992) to develop hypotheses of historical distributions based on projections for 6000 years before present (6 ka BP) and 21 ka BP climatic scenarios. Data files were downloaded at their original 2.8125° resolution and resampled to 30'' resolution via bilinear interpolation.

To convert the continuous probabilities and outputs yielded by MAXENT and BIOCLIM palaeoclimate projections into presence/absence maps, we applied the threshold values that maximized Kappa under current climatic conditions. A map of areas showing historical stability (as Hugall *et al.*, 2002) was generated for each forest definition by summing the presence/

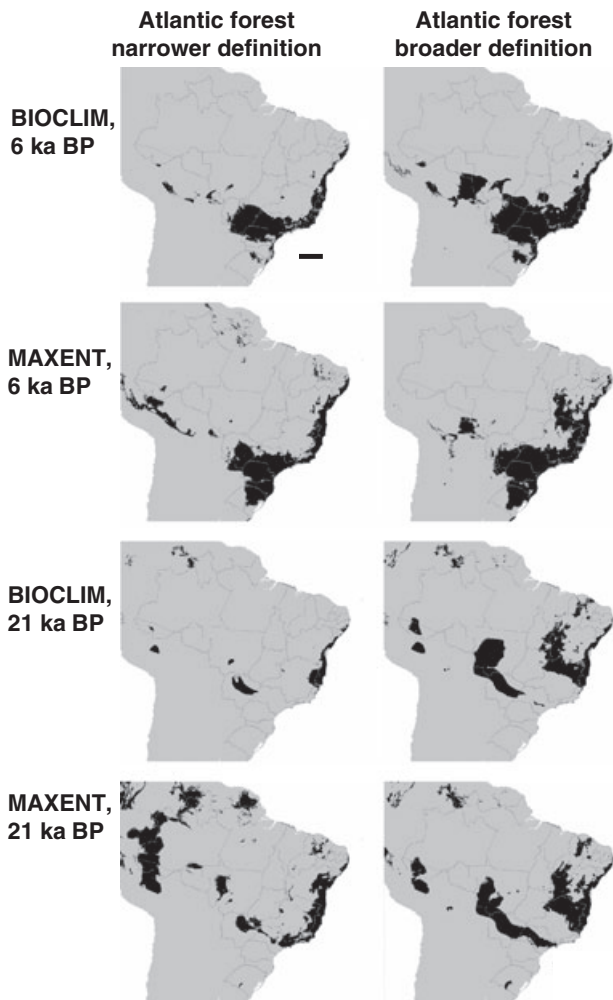


Figure 2 Modelled ranges of the Atlantic forest under narrow and broader definitions, 6 ka BP, and 21 ka BP climate using BIOCLIM and MAXENT modelling approaches. Scale bar = 500 km.

absence maps obtained under current, 6 ka BP and 21 ka BP projections yielded by both BIOCLIM and MAXENT models. This combined area map depicts areas which were potentially occupied by forests during the climatic oscillations of the late Pleistocene and Holocene. Refugia or historically stable areas were defined as those grid cells for which forest presence was inferred in all models and time projections.

Palaeoclimatic model validation and comparisons with patterns of endemism and with phylogeographical data

Predicted past ranges were qualitatively evaluated against vegetation types inferred from pollen records at sites located within the current or predicted range of the Atlantic forest, as well as in areas currently occupied by the neighbouring Cerrado – a savanna-like biome. To that end, we used all available pollen data dating from or around the 6 ka BP and

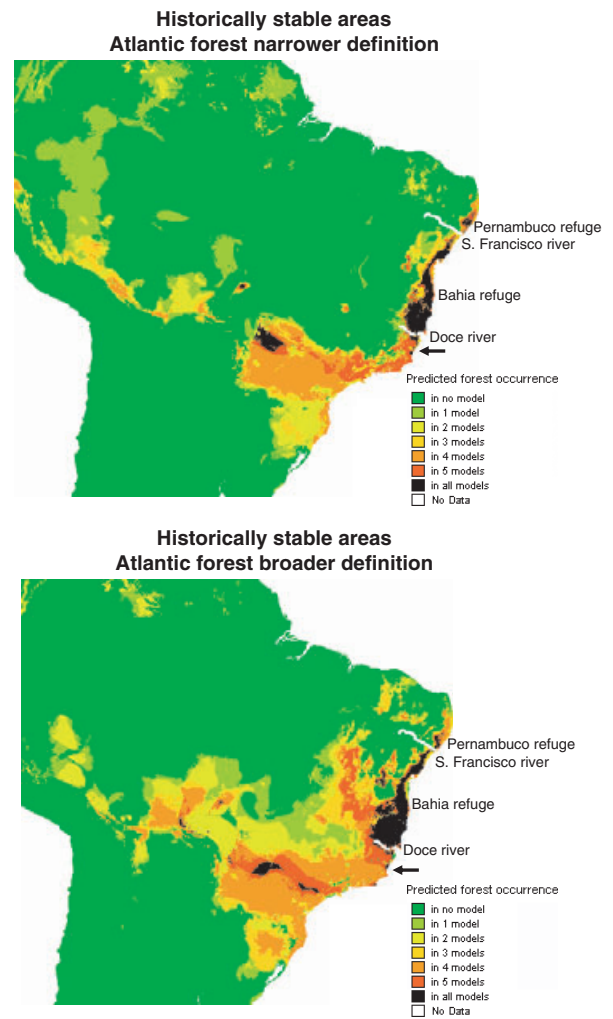


Figure 3 Summary maps of historically stable areas for the Atlantic forest under narrow and broader definitions, obtained by summing across BIOCLIM and MAXENT output grids for forest absence/presence under current, 6 ka BP and 21 ka BP climatic scenarios. White lines depict the São Francisco (top) and Doce (bottom) rivers. The arrow indicates the small refuge predicted at the boundaries of the states of Espírito Santo and Rio de Janeiro.

21 ka BP periods between latitude 3° S and 33.7° S and longitude 58° W and 34.8° W, excluding sites located in the Amazonian forest biome. For the 6 ka BP period, records were available from 11 localities, representing 10 spatially unique points at 30'' resolution; for the 21 ka BP period, data existed for four sites (Tables 1 & 2, Fig. 4).

Hypothesized refuge areas were compared with published patterns of species diversity and endemism for birds (da Silva *et al.*, 2004), mammals (Costa *et al.*, 2000), butterflies (Tyler *et al.*, 1994), bamboo species (Soderstrom *et al.*, 1988) and woody plants (Prance, 1982). They were also contrasted with molecular data available for the local fauna (see below). In both instances, we would expect to find evidence of high species endemism and high genetic diversity in major areas of predicted stability (refugial zones) and, in contrast, lower

Table 1 Comparison between published pollen records and model predictions for forest occurrence at 6 ka BP as shown in the stability area map of Fig. 4(a). Numbers refer to localities shown in Fig. 4(a).

Locality	Latitude, longitude, altitude	Information from pollen data	Reference	Model prediction	Match between model and data?
1. Lago do Pires, State of Minas Gerais	17°57' S, 42°13' W, 390 m	7500–5530 yr BP: reduced gallery forest elements; presence of <i>Curatella</i> pollen indicates savanna-type vegetation	Behling (1998)	Outside area of forest stability at 6 ka BP	Yes
2. Lagoa Nova, State of Minas Gerais	17°58' S, 42°12' W, 390 m	6060–2180 yr BP: expansion of semi-deciduous forest components in area previously occupied by savanna-type vegetation	Behling (2003)	Outside area of forest stability at 6 ka BP. However, both models of the forest under broader definition at 6 ka BP predict forest occurrence at this site	No when compared with summary map; Yes when compared with maps of forest under broader definition
3. Jacareí, State of São Paulo	23°17' S, 45°58' W, 550 m	8240–5400 yr BP: arboreal pollen present; presence of <i>Coccoloba</i> pollen indicates forest-type vegetation	Garcia <i>et al.</i> (2004)	Within area of forest stability at 6 ka BP	Yes
4. Serra da Boa Vista, State of Santa Catarina	27°42' S, 49°09' W, 1160 m	10,000–3000 yr BP: highland (more inland) sites show predominance of grassland vegetation	Behling (1995, 1998)	Outside area of forest stability at 6 ka BP. Near forest boundary	Yes
5. Fazenda do Pinto, São Francisco de Paula, State of Rio Grande do Sul	29°24' S, 50°34' W, 900 m	6630 ± 195 yr BP: increase of Weinmannia-type pollen indicates afforestation	Behling <i>et al.</i> (2001)	Outside area of forest stability at 6 ka BP	Yes
6. Morro da Igreja, State of Santa Catarina	28°11' S, 49°52' W, 1800 m	7500–4000 yr BP: deposition gap attributed to dry environment, related to absence of humid forests	Behling (1995, 1998)	Outside area of forest stability at 6 ka BP	Yes
7. Serra do Rio Rastro, State of Santa Catarina	28°23' S, 49°33' W, 1420 m	10,000–3000 yr BP: predominance of grassland, but other arboreal components also abundant	Behling (1995, 1998)	Outside area of forest stability. Near forest boundary at 6 ka BP	Yes
8. Morro de Itapeva, State of São Paulo	22°47' S, 45°32' W, 1850 m	9900–2610 yr BP: increased representation of cloud forest taxa and <i>Araucaria</i> forest (as opposed to tropical rain forest elements)	Behling (1997a)	Outside area of forest stability at 6 ka BP. Near forest boundary	Yes
9. Colônia, State of São Paulo	23°52' S, 46°42' 20" W, 900 m	8000–0 yr BP: increase in percentage of arboreal pollen	Ledru <i>et al.</i> (2005)	Outside area of forest stability. Near forest boundary at 6 ka BP	Yes
10. Salitre, State of Minas Gerais	19° S, 46°46' W, 1050 m	8000–5000 yr BP: replacement of <i>Araucaria</i> forest by semi-deciduous forest	Ledru (1993)	Outside area of forest stability at 6 ka BP. However, both models of the forest under broader definition at 6 ka BP predict forest occurrence at this site	No when compared with summary map; Yes when compared with maps of forest under broader definition
11. Serra Campos Gerais, State of Paraná	24°40' S, 50°13' W, 1200 m	9660–2850 yr BP: predominance of grassland (73–93%), but tropical rain forest taxa increasing (3–9%)	Behling (1997b)	Outside area of forest stability. Near forest boundary at 6 ka BP	Yes

Table 2 Comparison between published pollen records and model predictions for forest occurrence at 21 ka BP as shown in the stability area map of Fig. 4b (unless otherwise specified). Numbers refer to localities shown in Fig. 4b.

Locality	Latitude, longitude, altitude	Information from pollen data	Reference	Model prediction	Match between model and data?
1. Catas Altas, State of Minas Gerais	20°05' S, 43°22' W, 755 m	48–17 ka BP: abundant grassland pollen taxa; low <i>Araucaria</i> forest pollen percentage; tropical rain forest pollen absent or very rare; fern spores sparse	Behling & Lichte (1997)	Outside area of forest stability at 21 ka BP	Yes
2. Colônia, State of São Paulo	23°52' S, 46°42'20" W, 900 m	24,615–19,670 yr BP: marked decrease in the proportion of arboreal pollen, going from frequencies of 53–80% around 24 ka BP to 4–14% around 19 ka BP	Ledru <i>et al.</i> (2005)	Outside area of forest stability at 21 ka BP	Yes
3. Morro de Itapeva, State of São Paulo	22°47' S, 45°32' W, 1850 m	35–17 ka BP: expansion of elevation grassland elements; absence of <i>Araucaria</i> forest, cloud forest, or Atlantic rain forest elements	Behling (1997a)	Outside area of forest stability at 21 ka BP	Yes
4. Salitre, State of Minas Gerais	19° S, 46°46' W, 1050 m	28,740–16,800 yr BP: sedimentation apparently interrupted	Ledru (1993)	Outside area of forest stability at 21 ka BP	Yes

diversity, lower endemism and molecular signatures of recent range expansion within species in unstable, recently recolonized regions (non-refugial areas).

Phylogeographical validation of model hypotheses used diversity patterns from published mitochondrial DNA (mtDNA) data. Because phylogeographical studies of the Atlantic forest fauna are in their infancy and most have limited sampling north of the Doce river, we were able to evaluate published mtDNA data from only five widespread taxa: the three-toed sloth *Bradypus variegatus* (de Moraes-Barros *et al.*, 2006), lizards of the *Gymnodactylus darwini* complex (Pellegrino *et al.*, 2005), the slender mouse opossum *Marmosops incanus* (Mustrangi & Patton, 1997), the four-eyed opossum *Metachirus nudicaudatus* (Costa, 2003) and the Atlantic tree rat *Phyllomys pattoni* (Leite, 2003). *Gymnodactylus darwini*, *M. incanus* and *P. pattoni* are restricted to the Atlantic forest biome (Vanzolini, 1953; Mustrangi & Patton, 1997; Freire, 1998; Leite, 2003). *Bradypus variegatus* also occurs in areas of central Brazil (de Moraes-Barros *et al.*, 2006). *Marmosops nudicaudatus* is distributed throughout Central and South American forests (Costa, 2003).

DNA sequences were downloaded from GenBank or obtained from authors (see Appendix S1 in Supplementary Material) and analysed in DNAsp (Rozas *et al.*, 2003) to compare levels of genetic diversity in refuge and non-refuge areas as defined by the models. We estimated nucleotide diversity (Lynch & Crease, 1990) and the mutation parameter (theta per site) based on the number of segregating sites (Watterson, 1975). We also used coalescent-based tests of

historical population expansion in non-refuge areas (Ramos-Onsins & Rozas, 2002). For the purposes of visualization, we plotted the distribution of observed pair-wise nucleotide site differences (mismatch distribution) and the expected distribution under demographic expansion for hypothesized refugial and non-refugial areas (Rogers & Harpending, 1992). Because the southern populations of *G. darwini* showed a mismatch distribution consistent with that expected under a scenario of population expansion and had sufficient variability, we also estimated the approximate date of population growth in Arlequin version 3.01 (Schneider *et al.*, 2000). Following Zamudio & Greene (1997), calculations were carried out with both low (0.47% sequence divergence per million years) and high (1.32% per million years) estimates of substitution rates.

RESULTS

Model predictions

Under current climatic conditions, AUC and maximum Kappa values indicated satisfactory model performance across methods and data sets (Fig. 1). Nevertheless, both BIOCLIM and MAXENT slightly over-predicted forest presence in interior areas of Brazil, which are occupied by distinct biomes such as the Cerrado and the wetlands of the Pantanal. Model accuracy was consistently higher in MAXENT when compared with BIOCLIM, and better performance was achieved for models of the forest under a narrow definition in comparison with models of the forest in the broadest sense (Fig. 1). Most of the

discordance among model outputs related to the proportion of over-predicted areas, which was greater in BIOCLIM maps and in models of the forest in its broadest sense. Predicted distributions under the 6 ka BP climatic scenario were not strikingly different from current distribution predictions, yet forest ranges were predicted to have undergone significant reduction within the cool dry period of 21 ka BP (Figs 1 & 2).

With regard to refugial areas predicted within the current (pre-anthropogenic clearing) range of the Atlantic forest, stability surfaces consistently predicted a large stable region stretching along the central corridor of the Atlantic forest, extending from the southern border of the Doce river northward to the southern border of the São Francisco river (Fig. 3). This will be referred to as the Bahia refuge throughout this paper. Another smaller refugial area was inferred north of the São Francisco river, referred to hereafter as the Pernambuco refuge. Remarkably, however, much of the area currently forested and located south of the Doce river was not predicted as suitable for forests under 21 ka BP conditions, irrespective of forest definition (Figs 2 & 3). Accordingly, this region was not

predicted to have retained a large, stable habitat area for forest-dependent species, but rather small and medium-sized refuges only.

Palynological validation of model outputs

The predictions provided by the intersection of 6 ka BP models generated by BIOCLIM and MAXENT (Table 1, Fig. 4a) were consistent with all pollen records available for this period. Lago do Pires, Serra da Boa Vista, Fazenda do Pinto, Morro da Igreja and Morro de Itapeva were predicted to be outside the rain forest area (Fig. 4a). Accordingly, their pollen records show evidence of reduced gallery forest, increased grassland or savanna components, a sedimentation hiatus probably due to dry conditions or increased cloud forest (cool weather adapted, as opposed to tropical rain forest) elements (Behling, 1997a, 1998, 2003; Table 1). Jacareí was predicted to be within the area of forest occurrence, and the pollen record indicates the presence of arboreal elements represented primarily by *Coccoloba*, a common tree in Brazil’s coastal forests (Garcia *et al.*,

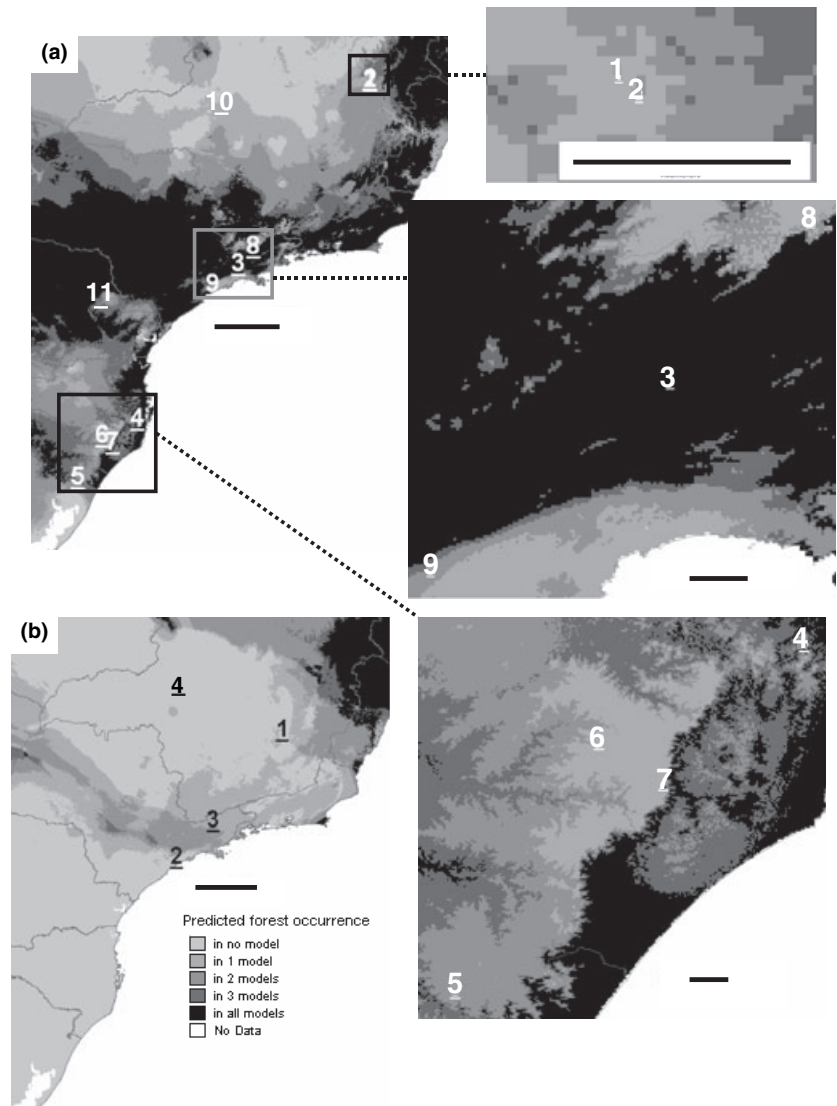


Figure 4 (a) Summary map adding all BIOCLIM and MAXENT 6 ka BP projections for both forest definitions. Localities with available pollen data are depicted: 1, Lago do Pires; 2, Lagoa Nova; 3, Jacareí; 4, Serra da Boa Vista; 5, Fazenda do Pinto; 6, Morro da Igreja; 7, Serra do Rio Rastro; 8, Morro de Itapeva; 9, Colônia; 10, Salitre; 11, Serra Campos Gerais. See Table 1 for locality details. Scale bar = 200 km (top left), 20 km (top, centre and bottom right). (b) Summary map adding all BIOCLIM and MAXENT 21 ka BP projections for both forest definitions. Localities with available pollen data are depicted: 1, Catas Altas; 2, Colônia; 3, Morro de Itapeva; 4, Salitre. See Table 2 for locality details. Scale bar = 200 km.

2004). Colônia, Serra do Rio Rastro and Serra Campos Gerais were predicted to be outside the forest area but near the forest boundary (Fig. 4a). For Serra do Rio Rastro, the palaeorecord shows a system dominated by grasses, with other arboreal components also being abundant (Behling, 1995, 1998) – matching the prediction of a forest–savanna ecotone. At Serra Campos Gerais, there is a predominance of grassland vegetation (73–93%), but tropical rain forest taxa show a trend of increasing representation (Behling, 1997b). Colônia is located within the Atlantic forest ecotone today, suggesting that the forest boundary depicted in Fig. 4a has progressively migrated south. This is in agreement with the pollen record at this site, which shows a general trend towards forest expansion at 6 ka BP (Ledru *et al.*, 2005).

For Lagoa Nova and Salitre, the literature specifically mentions an increase of semi-deciduous elements (Ledru, 1993; Behling, 2003). The 6 ka BP summary map (Fig 4a) did not coincide with these observations. However, this was expected because the map represents a summation of occurrence predictions for the forest as defined both narrowly (that is, ignoring the semi-deciduous vegetation) and broadly (including semi-deciduous components). For a valid comparison, Behling's (2003) and Ledru's (1993) observations should be contrasted with predictions of the distribution of the forest under a broad definition only (Fig. 2). When this is done, we observe complete agreement between the models and the pollen data (not shown).

Three pollen data sets that bracket or approximate the period of 21 ka BP (Colônia, Morro de Itapeva, Catas Altas)

indicate expansion of grassland vegetation and/or low percentage of pollen from humid tropical vegetation, hence agreeing with the predictions from the 21 ka BP consensus map (Table 2, Fig. 4b). The records are in agreement with predicted absence of forest in BIOCLIM models (under both forest definitions), but conflict with the projections made by MAXENT, which predicted the presence of forest in two areas. The latter approach therefore appears to over-predict forest distribution during the 21 ka BP period (Figs 2 & 4b). In Salitre, there is an apparent sedimentation gap around this time (Ledru, 1993; Table 1), which could be associated with dry conditions.

Comparison with published mtDNA sequences

Populations from the hypothesized Bahia refugial area showed higher mtDNA diversity than non-refugial areas located to the south or west for three of the five species (Table 3): the sloth (*B. variegatus*), the lizard (*G. darwini*) and the broad-ranging marsupial (*M. nudicaudatus*). By contrast, southern populations of both the slender mouse opossum (*M. incanus*) and the Atlantic rat (*P. pattoni*) had higher collective diversity than those located within the range of the hypothesized Bahia refuge. Recent population expansions were not inferred for the southern (non-refugial) region in any of the species. However, *B. variegatus* and *G. darwini* showed sequence mismatch distributions that fitted well with expected curves under a hypothesis of population growth, whereas *M. nudicaudatus*, *M. incanus* or *P. pattoni* failed to do so (Fig. 5).

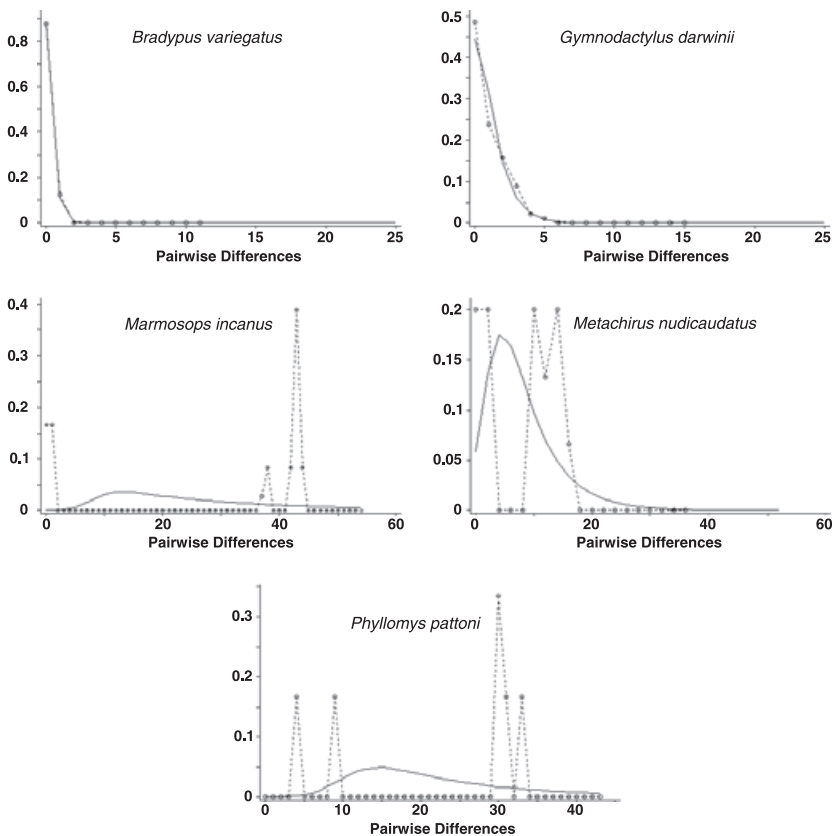


Figure 5 Mismatch distributions of mtDNA sequences from non-refugial areas as per model predictions, based on available molecular data sets. Observed frequencies of pairwise differences (dotted line) are compared against expected frequencies under a scenario of expanding population sizes (continuous line).

Table 3 Analyses of mtDNA sequence data available for Atlantic forest species. Given are sample size (*n*), two measures of genetic diversity (Pi and Theta), and the results for the population expansion test (*R*₂).

Species	Marker, size	Predicted area as per climatic models	Locality representation in original dataset	Nucleotide diversity, Pi	Theta per site	<i>R</i> ₂ (<i>P</i> -value)	Reference; source of sequences
<i>Bradydipus variegatus</i>	mtDNA, control region, 362 bp	Bahia refuge Non-refugial area	SE Bahia, N Minas Gerais São Paulo	26 0.00182 16 0.00035	0.00217 0.00083	0.1162 (<i>P</i> = 0.340) 0.2421 (<i>P</i> = 0.820)	de Moraes-Barros <i>et al.</i> , (2006); GenBank
<i>Gymnodactylus darwini</i>	mtDNA, cytochrome <i>b</i> , 461 bp	Bahia refuge Non-refugial area	SE and NE Bahia, Espírito Santo São Paulo	13 0.10298	0.07301	0.2265 (<i>P</i> = 0.996)	Pellegrino <i>et al.</i> (2005); GenBank
<i>Marmosops incanus</i>	mtDNA, cytochrome <i>b</i> , 400 bp	Non-refugial area Bahia refuge Non-refugial area	São Paulo Bahia, N Espírito Santo Rio de Janeiro, São Paulo, Minas Gerais	20 0.00208 21 0.03571 24 0.06093	0.00429 0.03552 0.04543	0.1050 (<i>P</i> = 0.215) 0.1378 (<i>P</i> = 0.610) 0.1761 (<i>P</i> = 0.948)	Musturangi & Patton (1997); J. Patton
<i>Metachirus nudicaudatus</i>	mtDNA, cytochrome <i>b</i> , 450 bp	Bahia refuge Non-refugial area	Bahia, Espírito Santo Minas Gerais, Rio de Janeiro, São Paulo	06 0.01613 06 0.00874	0.01200 0.00973	0.2944 (<i>P</i> = 0.932) 0.1667 (<i>P</i> = 0.237)	Costa (2003); J. Patton
<i>Phyllomys pattoni</i>	mtDNA, cytochrome <i>b</i> , 1021 bp	Bahia refuge area Non-refugial area	Bahia, Espírito Santo Minas Gerais, Rio de Janeiro	13 0.01155 04 0.02356	0.01365 0.02083	0.1167 (<i>P</i> = 0.175) 0.2376 (<i>P</i> = 0.486)	Leite (2003); J. Patton

For *G. darwini* the time of population expansion is estimated at approximately 1 million generations ago under the assumption of 0.47% mtDNA divergence per million years (95% CI 0–3,383,486), and of roughly 350,000 generations under a 1.32% divergence rate (95% CI 0–1,204,726). Given the wide confidence intervals, we are unable to discriminate between late or mid-Pleistocene events for this species.

DISCUSSION

Based on evaluation statistics, MAXENT models under current climate were more accurate than BIOCLIM projections – which is in agreement with recent comparative studies across modelling techniques (Elith *et al.*, 2006). Both approaches nonetheless predicted forest occurrence in areas of interior Brazil that are occupied by the Cerrado. Such over-prediction must not necessarily be interpreted as poor performance, as the models employed climatic data alone to predict the potential distribution of the Atlantic forest, thus ignoring other factors that may play a role in restricting its realized distribution (e.g. soil, topography, fire). In agreement with Hijmans & Graham (2006), the pollen records indicated that MAXENT projections under 21 ka BP climatic conditions overestimated the range of the forest for that period, reinforcing the value of combining multiple models in biodiversity prediction (Thuiller, 2004; Araújo & New, 2007).

The 11 published pollen records available for the target area during the 6 ka BP period are consistent with the multi-model inference of forest distribution. These results are encouraging even after acknowledging the limited fossil data and the potential pitfalls associated with the use of the pollen record. Behling *et al.* (1997) showed that modern pollen rain can vary in Atlantic forest sites and may fail to represent all plant species locally present, reinforcing the need for a careful interpretation of the fossil pollen record. Assuming that the palaeorecords used here provide a reasonably accurate picture of Brazil’s past flora, we can affirm that the best fossil pollen information available to date matches the predictive models of Atlantic forest refugia.

Abundant data about the Amazonian pollen record, palaeoclimate and phylogeography point to a continuous presence of mesic forest throughout the last ice age and falsify the refugia hypothesis as a major process of regional biological diversification (Colinvaux *et al.*, 2000; Lessa *et al.*, 2003; Bush *et al.*, 2004; Mayle *et al.*, 2004; Urrego *et al.*, 2005; Bush & de Oliveira, 2006). Although data are much more sparse for the Atlantic forest, it has been proposed that climatic fluctuations and the impact of Pleistocene arid phases were greater in the southern portion of the biome when compared with the northern region (Por, 1992). This is consistent with the palaeomodels presented here. However, it has also been suggested that the Atlantic forest was able to maintain a reasonable degree of continuity during such climatic events, thanks to the complex topography of the region and the potential for vertical migration (Por, 1992). The climatic modelling of historically stable areas suggests a different

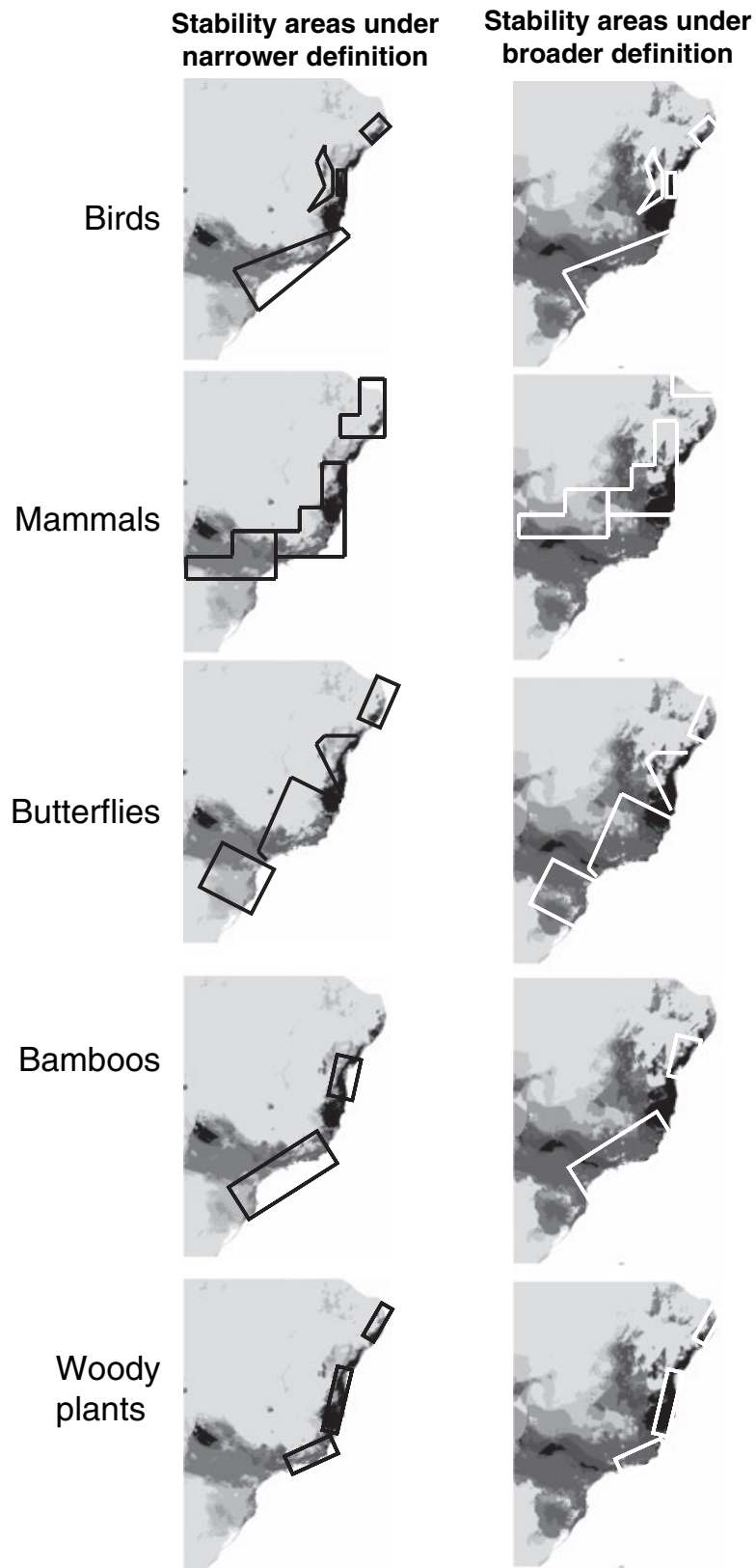


Figure 6 Comparisons between predicted areas of historical stability of the Atlantic forest and centres of endemism previously proposed for birds, mammals, butterflies, bamboos and woody plants. Left and right columns depict stability maps of the forest under narrower and broader definitions, respectively, as in Figure 3. Boxes indicate the location of centres of endemism proposed for each taxon according to the literature.

scenario, predicting forest contraction in southern and south-eastern Brazil around 21 ka BP and the existence of a large Bahia forest refuge, as well as a Pernambuco refuge, along the north-eastern Brazilian coast. The presence of major refugial areas covering most of the central and northern range of the Atlantic rain forest contrasts strongly with the relative instability of forest habitats predicted south of the Doce river, suggesting strikingly different Quaternary histories from a spatial perspective. This view deserves further testing but is supported by existing pollen records, which indicate expansion of grassland elements, decrease in the proportion of arboreal pollen, decrease or absence of tropical rain forest pollen or apparent sedimentation interruption (Ledru, 1993; Behling, 1997a; Behling & Lichte, 1997; Ledru *et al.*, 2005). Two recent phylogeographical studies of Atlantic forest taxa distributed south of the Doce river – lancehead pitvipers of the *Bothrops jararaca* complex (Grazziotin *et al.*, 2006) and the woodcreeper, *Xyphorhynchus fuscus* (Cabanne *et al.*, 2007) – also demonstrated demographic changes consistent with responses to Pleistocene forest contractions and subsequent advances into southern areas of the Atlantic biome in response to late Quaternary climate change. When tied to the palaeoclimatic predictions presented here, this raises a hypothesis of a dynamic Pleistocene period in coastal Brazil, where modification in Atlantic forest cover or composition was tracked by the demography of the local taxa. Pellegrino *et al.* (2005) suggested rivers as underlying factors behind currently observed patterns of biological diversity in Brazil. Our study shows that at least two of the rivers referred to by their paper (Doce and São Francisco) are located at the boundaries of forest refugia suggested by the climatic models, thus providing an alternative to the riverine-barrier hypothesis (Moritz *et al.*, 2000).

Palaeomodelling does have predictive value relative to species endemism and phylogeography in Brazil's Atlantic forest. Analyses of species distribution employing various organisms and analytical methods (Prance, 1982; Soderstrom *et al.*, 1988; Tyler *et al.*, 1994; Costa *et al.*, 2000; da Silva *et al.*, 2004) are remarkably similar in pointing out distinct Bahia and Pernambuco centres of endemism in north-eastern Brazil (Fig. 6). The recent discovery of a new genus of microteiid lizard endemic to the southern Bahia region (Rodrigues *et al.*, 2005) reinforces previous evidence of the uniqueness of this relatively unexplored region. Three out of the five available mtDNA data sets for widespread taxa also conformed to an expectation of higher diversity in northern refugial areas. Moreover, the sloth and the lizard molecular data showed trends of population expansion in non-refugial localities.

Deforestation rates in north-eastern Brazil have been significantly higher than observed elsewhere along the coast (Fundação SOS Mata Atlântica, 1992). Increasing attention to conservation and population monitoring has recently been given to Pernambuco's centre of endemism (da Silva & Tabarelli, 2000, 2001; Tabarelli & da Silva, 2002; Tabarelli & Peres, 2002; Uchoa Neto & Tabarelli, 2002; Tabarelli *et al.*,

2004). However, there have been relatively fewer conservation measures and studies around the Bahia refuge area, especially when compared with regions located south of the Doce river. Because refuge modelling is proving effective for predictions of patterns of species and genetic endemism in Atlantic forest taxa (e.g. sloths, lizards, pitvipers, woodcreepers), as well as for low-dispersal rain forest specialist taxa elsewhere (Graham *et al.*, 2006), we predict that further sampling of the under-explored Bahia refuge area will reveal high endemism at both species and genetic levels.

In contrast to Por's (1992) suggestion of Atlantic forest retention during the climatic tribulations of the Pleistocene, the palaeoclimatic models failed to predict the persistence of large forests in areas of mid to high elevation south of the Doce river. As a corollary, the wide-ranging southern areas of endemism depicted by da Silva *et al.* (2004), Costa *et al.* (2000), Tyler *et al.* (1994), Soderstrom *et al.* (1988) and Prance (1982) encompass only a few small-sized potential refugia as inferred from the modelled stability surfaces. Likewise, the patterns of molecular phylogeography in *M. incanus* and *P. pattoni* conflict with the model predictions, showing higher molecular diversity south of the Doce river. A possible explanation for such discord may involve suboptimal model performance in areas of steep environmental gradients, which are more common in south-eastern Brazil. This could have happened, for instance, due to additional moisture input from cloud interception, or if the bilinear interpolations used to resample the original palaeoclimatic surfaces failed to represent the true climatic estimates of sites at high altitudes or in valleys (Daly, 2006), thus affecting model performance in topographically complex areas. Therefore, it is possible that multiple patches of forest once existed in Brazil's south-eastern and southern regions but were not identified by our models. Another scenario to consider is that forest persisted in areas currently occupied by the Atlantic Ocean, which may have been possible through Pleistocene sea-level changes (Caruso *et al.*, 2000). Disagreements between modelled processes and observed genetic patterns can also be caused by taxon-specific features such as unique natural histories, limited habitat tolerance and current dispersal capabilities (Hugall *et al.*, 2002; Lara *et al.*, 2005; Graham *et al.*, 2006). It is important to keep in mind that the available molecular data have limited geographical coverage and small sample sizes relative to our model predictions (e.g. Costa, 2003; Leite, 2003), and low spatial resolution (e.g. Costa *et al.*, 2000). Further studies based on an experimental design appropriate for refuge testing are therefore needed.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 mtDNA sequences used in phylogeographical analyses.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2699.2007.01870.x> (This link will take you to the article abstract.)

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Ana Carnaval is an NSF post-doctoral fellow in the Moritz Lab. She studies phylogeographical patterns and their underlying historical processes in tropical forests, with emphasis on the Brazilian Atlantic forest. Her current research combines the use of climatic models and data on genetic and species diversity to promote insight about historical events, community response to future climate change and their implications for conservation.

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