

Phylogeography of vertebrates on the Sunda Shelf: a multi-species comparison

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ABSTRACT

Aim Pleistocene environmental fluctuations had well-characterized impacts on the patterns of within-species divergences and diversity in temperate habitats. Here we examine the impact the Pleistocene had on widely distributed forest vertebrates in a tropical system where the distribution of the habitat was affected by those fluctuations.

Location Sundaland, tropical Southeast Asia.

Methods We conducted a comparative phylogeographical analysis of 28 non-migratory, forest-dependent vertebrates, for which we constructed rooted, intraspecific phylogenies based on mitochondrial DNA sequences of individuals from at least the three major landmasses in the area (Borneo, Sumatra and the Malay Peninsula) and compared them to hypothetical phylogenies based on independent geological data and climate models regarding connections and relationships between the major landmasses of Sundaland. Java was included where possible. We dated the phylogenies to determine whether patterns of differentiation were concordant across species.

Results In most species, populations on the Malay Peninsula and Sumatra were most closely related, and sister to those from Borneo. The dates of these divergences, however, varied extensively between species. Borneo harbours multiple deeply divergent lineages of many species compared to the diversity within those species. Javan populations of several birds were most divergent relative to those from the rest of the Sunda Shelf.

Main conclusions These results suggest a dynamic history, including recurrent population extinctions and replacements and a strong priority effect for local populations. The close relationship between populations in Sumatra and the Malay Peninsula supports the existence of forest on the exposed shelf during the Pleistocene at many different times, and suggests that proximity was more important than the presence of palaeorivers for dispersal of forest taxa between landmasses.

Keywords

Comparative phylogeography, extinction, molecular dating, Pleistocene divergence, population divergence, priority effect, Sundaland.

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INTRODUCTION

Phylogeographical patterns have been used extensively to improve our understanding of the evolutionary history of individual species or species complexes across many taxa (e.g. González-Porter *et al.*, 2011; Koblmüller *et al.*, 2012; Den Tex & Leonard, 2014). Despite the large number of

phylogeographical studies, relatively few comparative phylogeographical studies have been undertaken (Bermingham & Moritz, 1998; Arbogast & Kenagy, 2001; Riddle *et al.*, 2008). Comparative phylogeographical studies have the potential to uncover the evolutionary history of ecosystems by simultaneously admitting spatial and temporal elements in the hypotheses (Arbogast & Kenagy, 2001), and uniquely allow

for biological replication in the form of the different species studied (Bermingham & Moritz, 1998). Some examples from tropical systems include the Australian tropical rain forest (Schneider *et al.*, 1998), the Guiana shield in the Amazon (Fouquet *et al.*, 2012), and most relevant to our study, Southeast Asia (Lim *et al.*, 2011). Each of these studies compared the phylogeography of six to 16 co-distributed species to test hypotheses associated with Pleistocene environmental changes that could have caused divergence. Widespread support for the importance of Pleistocene events in driving within species divergences was found in Australia and the Amazon, but tropical Southeast Asia yielded mixed results, with some Pleistocene and some earlier divergences. Pleistocene age divergences thought to be associated with environmental changes were also identified in comparative phylogenetic studies of five or six species in temperate regions (Carstens *et al.*, 2005; Qu *et al.*, 2010).

Here we applied comparative phylogeography to Sundaland, a biodiversity hotspot in tropical Southeast Asia that inspired Alfred Russel Wallace to conclude that species originated through natural selection (Wallace, 1858). The forest across this region is currently divided by sea. The three largest landmasses are the Malay Peninsula, Sumatra and Borneo, but there are also many other islands of various sizes, including Java (Fig. 1). Pleistocene climatic fluctuations led to repeated changes in sea level, resulting in the periodic connection of the landmasses across the shelf (Fig. 1).

Although there are many locally endemic species in this biodiversity hotspot (Myers *et al.*, 2000), there are also many species widespread across the region (Heaney, 1986). We used data from all non-migratory, forest-dependent vertebrates for which mitochondrial DNA data are available from at least Sumatra, Borneo and the Malay Peninsula to test the following hypotheses based on independent, geological data. The periodic land connections suggest the hypothesis that

there were panmictic populations across the region during these times. This is further supported by climatic reconstructions suggesting that both highland and lowland forests across Sundaland were more extensive in periods of glacial maxima (Cannon *et al.*, 2009). This predicts very recent or no divergence between populations (Fig. 2a). However, it has been hypothesized that the Pleistocene land bridges were much drier than the islands and were either completely or partially covered by grasslands or savanna (Heaney, 1991; Meijaard, 2003; Bird *et al.*, 2005; Wurster *et al.*, 2010). In this case, the land that connected what today is Borneo, Sumatra and the Malay Peninsula may have been as efficient a barrier as the sea for inhibiting gene flow within forest-dependent species. This would predict that genetic divergences between populations would be ancient, and geographical distances would be the primary factor determining relationships (Fig. 2b). Alternatively, it has been hypothesized that gallery forests along palaeorivers running through the xeric exposed land during periods of low sea level could have acted as corridors for forest-dependent taxa (Heaney, 1991; Voris, 2000), and thus would have led to a pattern of gene flow more associated with these routes than distance (Fig. 2c). The other possible way the populations of a species on the three landmasses could be related, with Borneo and the Malay Peninsula being most closely related, is illustrated in Fig. 2d.

MATERIALS AND METHODS

Materials

Data from all forest-dependent, non-migratory species for which mitochondrial DNA sequences were available from at least the Malay Peninsula, Sumatra and Borneo, were collected from the literature. Although Java is also a large

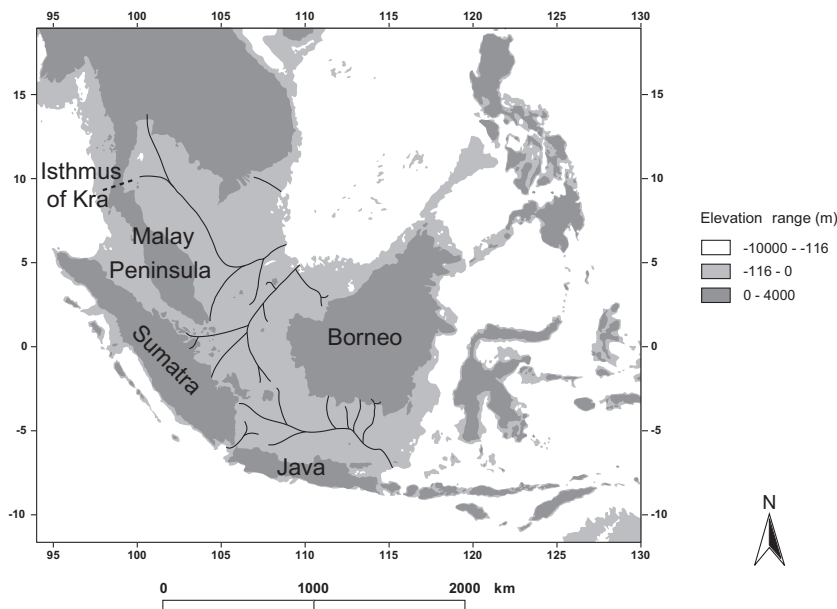


Figure 1 Map of Southeast Asia illustrating the positions of Borneo, Sumatra and Java relative to mainland Southeast Asia and the Malay Peninsula and the Isthmus of Kra. Current land is shown in dark grey, the maximum exposure of the Sunda Shelf (the -116 m contour line) is indicated with the light grey, and ocean is in white. Palaeorivers are shown as in Heaney (1991). Figure modified after Sathiamurthy & Voris (2006).

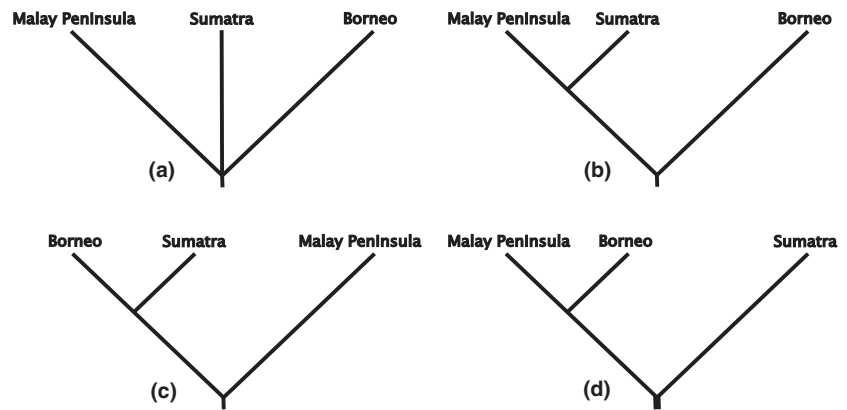


Figure 2 Four possible population phylogenies that illustrate the theoretical relationships between populations of a species distributed across the Malay Peninsula, Sumatra and Borneo.

island, it has been included in few phylogeographical studies of the region, probably because many widely distributed species are absent, perhaps because of its different climate (Wilting *et al.*, 2012). In total the data set comprised 31 taxa (Table 1), including 12 species of mammal [six rodents, two primates, one artiodactyl (*Sus barbatus*), one erinaceomorph (*Hylomys suillus*), one carnivoran (*Paradoxurus hermaphroditus*) and one proboscidean, the Asian elephant (*Elephas maximus*)], 17 species of bird [12 songbirds, four barbets and one kingfisher (*Ceyx erithaca*)], one flying lizard (*Draco sumatranus*) and one frog (*Rana chalconota* s.l.). Of these 31 taxa, three were excluded from further analysis because of taxonomic uncertainty due to lack of monophyly of the species: the erinaceomorph (*Hylomys suillus*), one primate (*Macaca nemestrina*) and a tree squirrel (*Sundasciurus tenuis*) (Ruedi & Fumagalli, 1996; Ziegler *et al.*, 2007; Den Tex *et al.*, 2010), leaving 28 species for analysis. Half of the species are endemic to Sundaland ($n = 15$), but some are also distributed north of the Isthmus of Kra (Table 1). It has been suggested in the literature that several of the species distributed beyond Sundaland may represent multiple species ($n = 5$; Table 1), indicating that the Sundaland populations may be distinct species. Within-species phylogenies included in the original publications were compared with the population phylogenies illustrated in Fig. 2 (see Table 1). The exception was the barbets because within-species phylogenies were not included in the original publication (Den Tex & Leonard, 2013). For this reason, within-species phylogenies with an appropriate outgroup for each species of barbet were constructed (see Appendix S1 in Supporting Information).

Dating

When available, dates from the original publication were used. Eleven data sets for which dates were not estimated in other publications (for four rodents *Leopoldamys sabanus*, *Maxomys whiteheadi*, *M. rajah* and *M. surifer*, the frog *Rana chalconota*, the lizard *Draco sumatranus*, the forktail *Enicurus leschenaulti*, and the four barbets *Megalaima rafflesii*, *M. henricii*, *M. chrysopogon* and *M. australis*) were tested for deviance from the molecular clock (Table 1). First, we used the Akaike information criterion (AIC) as implemented in MODELTEST 3.7

(Posada & Crandall, 1998) to obtain the most appropriate model of sequence evolution for the ingroup taxa. Then MEGA (Tamura *et al.*, 2013) or PAUP* 4b10 (Swofford, 2002) was used to obtain maximum likelihood (ML) scores for both the constrained and unconstrained trees. Lastly, the maximum likelihood ratio test was applied to the scores to test whether the molecular clock for each data set was violated.

Out of these 11 data sets, we were able to date nine: the four barbet phylogenies (Den Tex & Leonard, 2013), the four rodent phylogenies (Gorog *et al.*, 2004; Achmadi *et al.*, 2013) and the forktail phylogeny (Moyle *et al.*, 2005). For all rodents, the divergence at *cytb* third codon transversions used was the rate of 1.85% per million years (Myr) (as estimated in Den Tex *et al.*, 2010); for the barbets (*Megalaima* spp.) we used a rate of 1.88% (SD 0.23%) per Myr (Nabholz *et al.*, 2009); and for the forktail a rate of 2.5% (SD 1.15%) per Myr (Nabholz *et al.*, 2009). The date estimated is for the divergence between the sequences in the different populations, which is always older than the actual divergence between the taxa, introducing a bias towards older dates. Because of uncertainty regarding the mutation rates of the markers used in the flying lizard (*Draco sumatranus*) and the frog (*Rana chalconota* s.l.), *ND2* and *ND3*, respectively, we refrained from dating these phylogenies.

RESULTS

Phylogenetic patterns

In 16 of the 28 taxa analysed, Borneo was basal to Sumatra and the Malay Peninsula (Fig. 2b). No geographical structure was found in five taxa, and the relationships among the three landmasses were unresolved in six taxa (Fig. 2a). The last taxon, the macaque (*Macaca fascicularis*), had structure that was inconsistent with the pattern found in the other taxa. The macaques on the islands (Borneo and Sumatra) formed a reciprocally monophyletic clade with respect to all of the continental populations with mitochondrial DNA (Tosi & Coke, 2007). In this case the lineages found on Borneo, Sumatra and the Malay Peninsula do not share a most recent common ancestor (the Malay Peninsula lineages share a most recent common ancestor with the other mainland lineages).

Table 1 Species list of vertebrate taxa that show the distribution of at least the Malay Peninsula (MP), Sumatra and Borneo and for which genetic data are available. Species endemic to Sundaland (to Huxley's Line) are in bold. Also tabulated here are the mitochondrial sequences (mt gene) on which the respective taxon phylogenies and splitting events are based, and the estimated dates of divergences.

Taxon summary				Dating results	
Order	Taxon	Reference	Mt gene	Borneo (MP–Sumatra)	MP–Sumatra
Rodentia	<i>Leopoldamys sabanus</i> (Thomas, 1887) ^a	Gorog <i>et al.</i> , 2004	<i>cytb</i>	2.29	2.24
	<i>Maxomys whiteheadi</i> (Thomas, 1894)	Gorog <i>et al.</i> , 2004	<i>cytb</i>	0.75	0.29
	<i>Maxomys surifer</i> (Miller, 1900) ^b	Gorog <i>et al.</i> , 2004	<i>cytb</i>	1.51	0.94
	<i>Maxomys rajah</i> (Thomas 1894) [†]	Achmadi <i>et al.</i> , 2013	<i>cytb</i>	0.24	0.13
	<i>Sundasciurus hippurus</i> (Geoffroy, 1831)	Den Tex <i>et al.</i> , 2010	<i>cytb</i>	0.3	Latest Pleistocene
	<i>Sundasciurus tenuis</i> (Horsfield, 1824) [*]	Den Tex <i>et al.</i> , 2010	<i>cytb</i>		
Primates	<i>Macaca fascicularis</i> (Raffles, 1821) ^c	Tosi & Coke, 2007	<i>cytb</i>	Mainland–islands split 1:2; island relationships unresolved ¹	
	<i>Macaca nemestrina</i> (Linnaeus, 1766) [*]	Ziegler <i>et al.</i> , 2007	<i>cytb</i>		
Artiodactyla	<i>Sus barbatus</i> Müller, 1838	Larson <i>et al.</i> , 2007	D loop	No geographical structure	
Insectivora	<i>Hylomys suillus</i> Müller, 1840 ^{*,d}	Ruedi & Fumagalli, 1996	<i>cytb</i>		
Carnivora	<i>Paradoxurus hermaphroditus</i> (Pallas, in Schreber, 1777) ^e	Patou <i>et al.</i> , 2010	<i>cytb</i>	3.4	1.95
Proboscidea	<i>Elephas maximus</i> Linnaeus, 1758	Fernando <i>et al.</i> , 2003	D loop	Unresolved relationships ²	
Coraciiformes	<i>Ceyx erithaca</i> (Linnaeus, 1758)	Lim <i>et al.</i> , 2010	ND2	No geographical structure	
Piciformes	<i>Megalaima rafflesii</i> (Lesson, 1839)	Den Tex & Leonard, 2013	<i>cytb</i>	No geographical structure ³	
	<i>Megalaima henricii</i> (Temminck, 1831)	Den Tex & Leonard, 2013	<i>cytb</i>	0.53	0.085
	<i>Megalaima chrysopogon</i> (Temminck, 1824)	Den Tex & Leonard, 2013	<i>cytb</i>	0.91	0.11
	<i>Megalaima australis</i> (Horsfield, 1821) ^g	Den Tex & Leonard, 2013	<i>cytb</i>	0.23	0.075
Passeriformes	<i>Enicurus leschenaulti</i> (Vieillot, 1818) ^g	Moyle <i>et al.</i> , 2005	ND2	0.27	No geographical structure
	<i>Pteruthius flaviscapis</i> (Temminck, 1836) ^g	Reddy, 2008	<i>cytb</i>	Unresolved relationships ⁴	
	<i>Copsychus saularis</i> (Linnaeus, 1758) ^c	Lohman <i>et al.</i> , 2010	<i>cytb</i>	0.32	No geographical structure
	<i>Copsychus saularis</i> (Linnaeus, 1758) ^c	Sheldon <i>et al.</i> , 2009	ND2, COI	0.47	No geographical structure
	<i>Arachnothera longirostra</i> (Latham, 1790) ^c	Lohman <i>et al.</i> , 2010	<i>cytb</i>	Unresolved relationships ⁵	
	<i>Pycnonotus goiavier</i> (Scopoli, 1786) ^c	Lohman <i>et al.</i> , 2010	<i>cytb</i>	1.37	No geographical structure
	<i>Rhipidura javanica</i> (Sparrman, 1788) ^c	Lohman <i>et al.</i> , 2010	<i>cytb</i>	No geographical structure	
	<i>Hypothymis azurea</i> (Boddaert, 1783) ^c	Lim <i>et al.</i> , 2011	ND2	Unresolved relationships	
	<i>Orthotomus sericeus</i> Temminck, 1836	Lim <i>et al.</i> , 2011	ND2	No geographical structure	
	<i>Hypogramma hypogrammicum</i> Müller, 1843 ^g	Lim <i>et al.</i> , 2011	ND2	Unresolved relationships	
	<i>Stachyris erythroptera</i> (Blyth, 1842)	Lim <i>et al.</i> , 2011	ND2	3.9	No geographical structure
	<i>Stachyris poliocephala</i> (Temminck, 1836)	Lim <i>et al.</i> , 2011	ND2	1.03	0.34
	<i>Malacocincla malaccensis</i> (Hartlaub, 1844)	Lim <i>et al.</i> , 2011	ND2	2.55	Unresolved relationship
	Squamata	<i>Draco sumatranus</i> (McGuire & Heang, 2001)	McGuire & Heang, 2001	ND2	No dating performed
Anura	<i>Rana chalconota</i> group (Schlegel, 1837)	Stuart <i>et al.</i> , 2006	ND3	No dating performed	

*Excluded: not a natural group.

†Only haplotypes that form a monophyletic group included (as in Achmadi *et al.*, 2013).

^aDistributed across Sundaland and north of Isthmus of Kra, probably multiple species (Carleton & Musser, 2005; Lunde *et al.*, 2008).

^bDistributed across Sundaland, Philippines and north of Isthmus of Kra, probably multiple species (Aplin *et al.*, 2008).

^cDistributed across Sundaland, Philippines and north of Isthmus of Kra.

^dDistributed across Sundaland and north of Isthmus of Kra, probably multiple species (Ruedi & Fumagalli, 1996).

^eDistributed across Sundaland, Philippines and north of Isthmus of Kra, probably multiple species (Duckworth *et al.*, 2008).

^fDistributed across Sundaland and north of Isthmus of Kra, probably three species (BirdLife International, 2014).

^gDistributed across Sundaland and north of Isthmus of Kra.

¹From Tosi & Coke (2007).

²Substructuring of 'Pleistocene origin' from Fernando *et al.* (2003).

³'Ancient' split 0.23 Ma.

⁴MP–Sumatra–Borneo split 0.4 Ma from Reddy (2008).

⁵Substructuring of late Pleistocene age.

Java

Thirteen of the 28 species included in these analyses are also distributed on the large island of Java, which is adjacent to the south-east of Sumatra (see Fig. 1) and is also located on the Sunda Shelf. We were able to obtain data from Javan populations for eight taxa. In two cases the Javan population was most closely related to the neighbouring island of Sumatra (the rodent *Maxomys surifer* and the carnivoran *Paradoxurus hermaphroditus*), whereas the relationships among the Sumatran, Javan and Bornean populations of the macaque (Tosi & Coke, 2007) and the kingfisher (Lim *et al.*, 2010) were unresolved. In the other four cases (all birds), the Java populations were very divergent to Malay Peninsula, Sumatra and Borneo (*Megalaima australis*, *Enicurus leschenaulti*, *Arachnothera longirostra* and *Pteruthius flaviscapis*). The barbet *M. australis* distributed on Java and Bali were reciprocally monophyletic with respect to the Malay Peninsula, Sumatra and Borneo populations also with high support (see Fig. S1 in Appendix S1). The taxonomy of the shrike-babblers is uncertain (Rheindt & Eaton, 2009), but the population/species on Java is divergent from the population/species found on Borneo, Sumatra and the Malay Peninsula. In two other cases (*Megalaima henricii* and *M. chrysopogon*), the sister taxon is endemic to Java or Java and Bali (*M. armillaris* and *M. corvine*, respectively; Den Tex & Leonard, 2013).

Dating

A molecular clock could not be rejected for any of the phylogenies of the nine taxa (four rodents and five birds) tested here. Dates from previously published papers were used for the other six taxa.

All but one intraspecific splitting event dated within the Pleistocene. Only the rodent *Leopoldamys sabanus* showed divergence of Sumatra and the Malay Peninsula and those with Borneo before 2 Ma. The divergence between Borneo, Sumatra and the Malay Peninsula populations of the other

four rodents (*Maxomys surifer*, *M. whiteheadi*, *M. rajah* and *Sundasciurus hippurus*) ranged from latest Pleistocene to 1.51 Ma. Similarly, the divergence between Borneo, Sumatra and the Malay Peninsula populations of birds diverged between the latest Pleistocene and 1.8 Ma. Both thrushes (*Copsychus saularis* and *Enicurus leschenaulti*) also have earlier Pleistocene divergences on Borneo. This suggests no great difference in phylogeographical patterns in small terrestrial mammals and similarly sized birds. The populations of the large mammals (pigs and elephants) diverged most recently, in the latest Pleistocene. Polytomies or non-geographically structured clades in the barbet and the babbler (*Megalaima rafflesii* and *Pteruthius flaviscapis*) were inferred to be of late Pleistocene age.

Based on the data presented here, the most common biogeographical pattern identified was a close relationship between Sumatra and the Malay Peninsula, with Borneo more divergent. In those cases, the Sumatra and Malay Peninsula lineages diverged between the mid- and latest Pleistocene in 10 of 11 of the dated phylogenies except for the rodent *Leopoldamys sabanus*, which diverged much earlier. The times of divergence do not cluster around a particular glacial cycle or other geological event, such as the eruption of Toba 0.735 Ma (Fig. 3). In the subset of phylogenies where Java was also sampled, it often diverged earlier than the other landmasses ($n = 4$).

DISCUSSION

Phylogenetic patterns

We found substantial genetic structure among most animal populations surveyed here that are widely distributed across the Sunda Shelf. In the majority of cases where structure was identified between populations (16 out of 17), the Sumatra populations were more closely related to the Malay Peninsula populations, with the Bornean populations more divergent from the other two (Fig. 2b). Published phylogenies on other

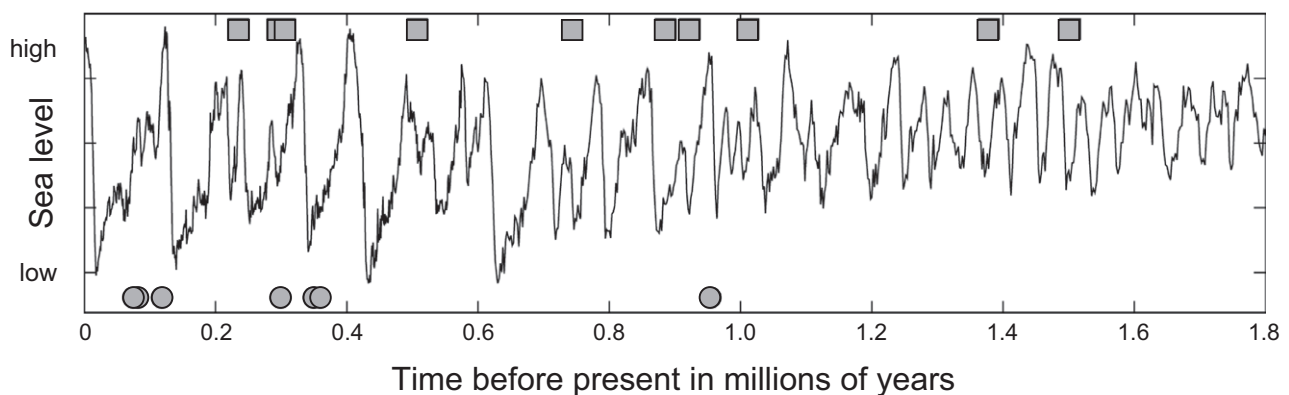


Figure 3 Age estimates of population splits within vertebrate species superimposed on the sea-level change curve during the Quaternary (0–1.8 Ma). The squares at the top represent the age estimates of the split of Borneo versus Sumatra and Malay Peninsula populations and the circles at the bottom indicate the age estimates of the split of the Sumatra versus Malay Peninsula populations, from Table 1. Proxy for sea-level changes after and modified from Lisiecki & Raymo (2005).

species distributed or sampled only on Borneo and Sumatra or the Malay Peninsula (but not both) also found deep divergences, such as the clouded leopard (*Neofelis nebulosa*), the orang-utan (*Pongo pygmaeus*) and the flying lemur (*Galeopterus variegatus*) (Steiper, 2006; Wilting *et al.*, 2007; Janečka *et al.*, 2008). The only exception to this pattern was the macaque (*Macaca fascicularis*), in which the Borneo and Sumatra populations form a clade, and the Malay Peninsula population is more closely related to the rest of mainland tropical East Asian macaques (Tosi & Coke, 2007). The other cases ($n = 11$) were unresolved or no geographical pattern was observed.

Borneo is not only most divergent from Sumatra and the Malay Peninsula. In several of the cases where multiple regions of Borneo were sampled, a large amount of divergence was identified between populations within Borneo (Moyle *et al.*, 2005; Den Tex *et al.*, 2010; Lim *et al.*, 2011; Den Tex & Leonard, 2013). This pattern is indicative of a long evolutionary history and warrants further investigation.

Java

Java contains fewer species than neighbouring (and larger) Sumatra, but relatively more of the species are endemic (MacKinnon & Phillips, 1993). This is probably a result of both history and environment. Java has a climate and vegetation that is different from the other islands on the Shelf (Wilting *et al.*, 2012). Many taxa that are widespread across Sundaland are absent from Java, such as tree squirrels of the genus *Sundasciurus* (Thorington *et al.*, 2012). The climate of Java is drier than in many other parts of the Sunda Shelf, and there are grassy areas as well as tropical forests (Heaney, 1991). In a few of the cases where data were available from Java as well as Sumatra, Borneo and the Malay Peninsula, the geographically close Sumatra was genetically most similar ($n = 2$). However, in most cases, specimens from Java represented the most divergent lineage ($n = 4$) or the sister species. We find it to be noteworthy that these within-species patterns were anticipated by some of Alfred Russel Wallace's observations of faunal similarity among the Malay Peninsula, Sumatra, Borneo and Java based on the distribution patterns of species and genera (Wallace, 1880, pp. 348–362), and his recognition of the importance of the shallow seas over continental shelf that separate these land areas.

Pleistocene divergence

Most intraspecific divergences, many of which represent named subspecies, date to the Pleistocene. Some closely related species/taxa, which have been referred to both as species and as subspecies, also diverged in the Pleistocene. This suggests that the Pleistocene had important environmental effects that impacted a wide variety of taxa. Our results indicate that the Pleistocene climatic/environmental fluctuations largely caused divergence and phylogeographical structure within species. This is similar to the pattern of Pleistocene

age intraspecific divergence found in Europe (Hewitt, 2000) and North America (Zink *et al.*, 2004). Through the Pleistocene, as the glaciers expanded, temperate Holarctic habitats receded into refugia. As the climate changed again and the ice melted, additional habitat became available to these temperate species, and the populations expanded. The pattern in Sundaland is the opposite of that for temperate forests. During glacial maximums sea level was lower, which exposed large parts of the Sunda shelf and potentially allowed a large expansion of both highland and lowland tropical forest in this region (Cannon *et al.*, 2009). These forests have a minimum extent in the interglacial periods, such as now (Woodruff, 2010).

Although the within-species divergence times occurred in the Pleistocene, they do not date back to the same time period (Fig. 3). This suggests that the common pattern was not caused by a single, perhaps particularly dramatic, climatic fluctuation or sea-level change, or another event such as a major volcanic eruption. Instead, this suggests that populations diverged and persisted for different periods of time, up to millions of years (Table 1). Eventually, some populations went extinct, in a somewhat stochastic fashion. At some point, surviving populations recolonized the areas where the species had gone locally extinct.

This scenario relies on the ability of forest-dependent taxa to expand out of their refugia and reach the other areas, which requires forest connections between Borneo, Sumatra and the Malay Peninsula on a relatively regular basis. Some forest-dependent taxa may be able to disperse across other habitats (especially the larger mammals), but we assume that this is less frequent than dispersal within forest (Heaney, 1986). When these forest taxa greatly expanded their ranges as more forest habitat became available across the Sunda Shelf, how could the observed common pattern arise? The rate of expansion may be much faster in areas where there is not already another population of the same species. This does not require that the divergent lineages stay isolated, just that the spread of genes is slowed down by the presence of other individuals already occupying the regions. This is similar to the ecological concept of 'priority effect', which is generally used in reference to a species' ability to survive and exclude other species, even when the other species may be better adapted to the environment, simply because they were there first (i.e. Mergeay *et al.*, 2011). In this case we refer to different populations of a single species. This is supported by the pattern of expansion out of refugia observed in many European temperate taxa (Hofreiter *et al.*, 2004).

CONCLUSIONS

Across a wide variety of forest-dependent vertebrates in Sundaland, populations on the Malay Peninsula and Sumatra are generally more closely related to one another than they are to populations on Borneo. The few cases where data were available from multiple populations on Borneo suggest that this landmass may harbour large amounts of genetic diversity

and interesting patterns of divergence which require further study based on better sampling. The different habitat available on Java may explain why only about half of the species studied here are distributed on this island. The birds from Java for which data were available suggest that the populations of these species on Java are the most divergent of the four landmasses. Although a common pattern was observed across many species, the timing of the divergences between populations on different landmasses varied widely and can not be attributed to a single event, such as a particularly extreme glacial cycle or massive volcanic eruption. The wide variation in dates suggests a more stochastic driver, resulting in different rates of population survival over tens of thousands to a few million years.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Phylogenetic reconstruction for the four species of barbet.

BIOSKETCH

This research group, headed by J.A.L., J.E.M. and R.T., is studying the ecology and evolution of the biodiversity of the Sunda Shelf, with a focus on forest-dependent vertebrates. This system is particularly well suited to study alternative hypotheses regarding differentiation and speciation in the tropics due to the geography and history of isolation and connection associated with Pleistocene climatic fluctuations. More information can be found at <http://www.consevol.org/>.

Author contributions: J.A.L. conceived the idea based on discussions with R.T. and J.E.M.; J.A.L. and R.-J.d.T. collected and analysed the data; and J.A.L. wrote the manuscript with support from all authors.

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