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Speciation dynamics in the Australo-Papuan *Meliphaga* honeyeaters

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Abstract

The Australo-Papuan *Meliphaga* honeyeaters have diversified over a wide range of habitats and elevational zones and are one of the few regionally known cryptic avian radiations. Using a combined 1580 bp of mitochondrial and nuclear DNA we investigate the species limits, systematic affinities and biogeographic history of *Meliphaga*. We also investigate the role of spatial sorting mechanisms, including altitudinal replacement and niche partitioning, as mechanisms underlying the adaptive radiation of this group. Phylogenetic analysis indicates that the genus *Meliphaga* comprises at least 16 species, three more than recognized in current classifications. The genus divides into two clades; the species-poor *lewinii* group, and the larger *analoga* group that has diversified into a wider range of vertical, vegetational and elevational niches. The basal division of each clade into an Australian and New Guinean assemblage was likely induced by the formation of the Arafura Sea during the early Pliocene (~4 MYA) with a single reinvasion of Australia by the open forest species *M. gracilis* during the early Pleistocene (1.2–1.5 MYA) via intermittent land bridges or island hopping. Most recent sister species were found to replace each other geographically within the same ecological and elevational zone conforming to the classical allopatric mode of speciation. In contrast, *M. orientalis* (650–1950 m) and *M. analoga* (0–1100 m) were found to replace each other altitudinally across ecological zones providing empirical support for altitudinal speciation as a mechanism of diversification in a montane avifauna. We find no evidence of sympatric speciation (co-existing sister lineages) and suggest that spatial segregation within the habitat (niche partitioning) is primarily a mechanism enabling more divergent species to coexist.

Keywords: *Meliphaga*; Altitudinal speciation; Biogeography; Phylogeny; New Guinea

1. Introduction

The Australo-Papuan region supports a rich endemic avifauna including radiations of the basal oscine lineages (Barker et al., 2002; Ericson et al., 2002). Within New Guinea and its adjacent island archipelagos much of this diversity has been attributed to speciation through isolation on ecological islands (oceanic islands and mountain tops) or separation across ecological barriers. Striking patterns of altitudinal replacement along the central cordillera and outlying ranges involving abrupt elevational transitions between species led Diamond (1972) to identify "montane speciation" as one of the most important mechanisms promoting diversification in the New Guinean avifauna. At lower altitudes (below 2000 m) where habitat complexity is at its greatest (Coates, 1985; Paijmans, 1976) species also exhibit a high degree of niche partitioning involving fine-scale differences in habitat preference, vertical zonation within the forest stratum and/or foraging ecology (Coates, 1985, 1990; Diamond, 1972). However, in the absence of information on the evolutionary relationships of the various geographical, altitudinal and ecological replacements it is difficult to correctly infer the mode(s) of speciation and patterns of adaptive radiation in the component avifaunas.

The dynamic Neogene paleoclimatic and geological history of New Guinea and surrounding regions is also expected to have had an impact on the mode and tempo of

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avian diversification. New Guinea occurs at the boundary of the Australian and Pacific/Caroline plates and is subject to ongoing tectonic change as these plates continue to interact at their margins (Hall, 2002; and references therein). This has resulted in considerable ongoing orogenic activity with accelerated mountain uplift during the Pliocene leading to a recent and rapid increase in the geographical breadth and ecological diversity of the region. The northern New Guinea margin continues to expand as adjacent islands (arc terranes) move westwards along the Pacific/Caroline Plate and collide with it in a process of strike-slip faulting (Hall, 2002; see also Pigram and Davies, 1987). This process of terrane accretion is ongoing and islands of the Bismarck Archipelago are expected to collide with New Guinea in the future. Less reliable is information concerning previous land connections between Australia and New Guinea. Flannery (1995; based on Dow, 1977) posits continuous separation of the landmasses during the Miocene with intermittent landbridges formed during the Pleistocene glacial periods. Others invoke Miocene and late Pliocene landbridges based on biogeographical patterns observed in Australo-Papuan radiations of mammals (Aplin et al., 1993) and snakes (Wüster et al., 2005) in conjunction with immunological or DNA clock estimates of divergence times, respectively. Coates (1985) also refers to a Miocene land connection following the formation of the Antarctic icesheet. Accumulating geological data indicate the presence of both Miocene and Pleistocene age landbridges (Bain and Draper, 1997; Langford et al., 1995) and the potential for periodic faunal inter-change between these regions.

Recent rapid uplift of the central cordillera in conjunction with the Pleistocene glacial cycles is predicted to have had significant impacts on climate and habitat changes in New Guinea and adjacent regions. This includes local aridification in southern lowland areas and increased rainfall in montane areas with significant cooling at higher altitudes (McAlpine et al., 1983). Pleistocene fluctuations caused vegetational zones to be lowered and compressed with a corresponding increase in the area of montane grasslands and lowland savannah (Hope et al., 2004).

The complex interaction between spatial, ecological, climatic and tectonic factors at different geographic scales, in conjunction with avian radiations characterised by complicated present-day distribution patterns of altitudinal, vertical and geographic displacement, suggest that phylogenetic investigation of patterns of speciation in the Australo-Papuan region would be revealing as to modes of speciation. The potential for different modes of speciation to occur at different geographic scales (Aleixo, 2004) and increasing interest in the role of speciation across ecological gradients or ecotones (Moritz et al., 2000; Schneider et al., 1999; Smith et al., 1997, 2001) in Neotropical (Aleixo, 2002) and Afrotropical (Smith et al., 2004) avifaunas provide a modern framework for comparative evolutionary studies of the avifaunas from these regions. Here, we use molecular phylogenetic approaches to investigate speciation processes in the endemic Australo-Papuan mimetic honeyeaters (Meliphagidae: *Meliphaga*).

The mimetic honeyeaters are a complex of mostly non-descript olive-brown birds that have diversified into a wide range of ecological niches in the Australo-Papuan region. The *Meliphaga* genus of 13 currently recognised species (Sibley and Monroe, 1990; Wolters, 1979) has its centre of distribution in New Guinea but ranges from Timor in the west (*M. reticulata*) to Tagula Island of the Louisiade Archipelago in the east (*M. vicina*), with a southern distributional limit in the temperate rainforests of south-east Australia (*M. lewinii*) (Fig. 1). The group is strikingly homogeneous in plumage with the principal diagnostic characters being the size and shape of the ear-spot, bill morphology and body size. In many cases diagnosis can only be made using a combination of characters. Certain vocalisations are shared between some species, and behavioural differences are often slight. Variation in plumage and size due to geography, age, sex and individual variation further confounds species identification in this genus (Coates, 1990).

In New Guinea, *Meliphaga* honeyeaters have diversified within the forests and savannah of the lowlands, foothills and mountains up to 2000 m. A number of species appear to replace each other geographically or altitudinally. However, ecologically and morphologically similar species occur in sympatry throughout parts of their range (Coates, 1990; Diamond, 1972; Schodde and Mason, 1999) where they appear to minimize competitive interactions with congeners by differences in bill morphology (presumably related to feeding ecology), microhabitat selection and patterns of vertical zonation. Although the presence of ecological and geographical sorting mechanisms is evident, the processes by which this genus diversified remain unclear.

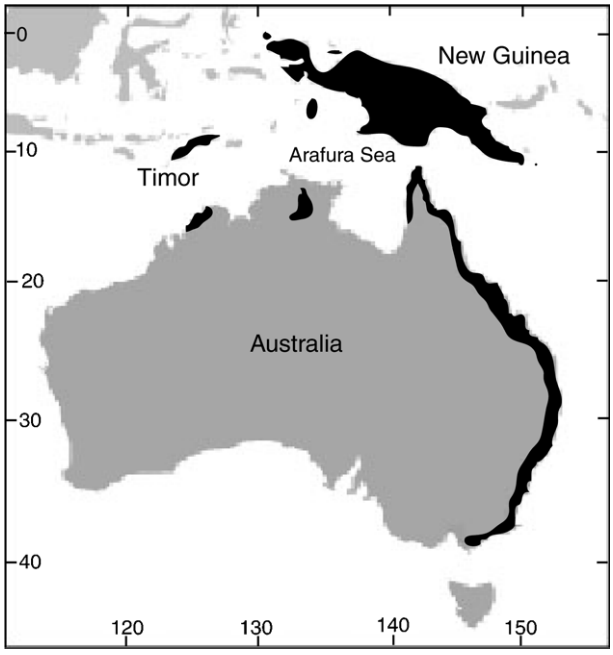

Fig. 1. Distributional limits of the Australo-Papuan *Meliphaga* honeyeaters.

Here, we use DNA sequence data from the mitochondrial (mt) NADH dehydrogenase subunit 2 (ND2) gene and nuclear β-fibrinogen intron 5 (FIB5) to assess species limits within *Meliphaga* and develop a phylogenetic hypothesis of relationships. Within this phylogenetic framework we explore the historical biogeography and mechanisms of adaptive radiation in the genus. In particular we assess the relative importance of different modes of speciation through assessment of geographical and ecological patterns of replacement amongst lineages. We test for:

(1) Classical allopatric speciation (Mayr, 1963) in which sister species occupy geographically disjunct ranges brought about by vicariance of ancestral habitats (dichopatric) or dispersal into new habitats (peripatric) (Bush, 1994).
(2) Ecological speciation in which sister species occupy distinctive adjacent habitats (parapatric) presumably as a result of divergence along environmental gradients or across ecotones (Endler, 1982; Moritz et al., 2000; Schneider et al., 1999; Smith et al., 1997, 2001). The altitudinal speciation model proposed by Diamond is considered a form of ecological speciation (Moritz et al., 2000).
(3) Sympatric speciation in which niche partitioning gives rise to co-distributed sister species.

2. Materials and methods

2.1. Taxon sampling

With the exception of the poorly known island endemic *M. vicina* from the Louisiade Archipelago, all currently recognised species of the genus *Meliphaga* (nomenclature following Coates, 1990; Sibley and Monroe, 1990) were included in our study. Where possible multiple representatives of each species were included to avoid the confounding effects of taxonomic mis-identifications and to assist in detecting the presence of cryptic variation. A limitation of this study, however, is that all New Guinean specimens are sampled from Papua New Guinea due to political difficulties of obtaining access to West Papua (formerly Irian Jaya). From the 49 specimens initially included in the study we subsequently identified a core dataset of 20 individuals to represent the taxonomic and genetic variation revealed within *Meliphaga*. Specimen details are listed in Appendix A. As outgroups, we obtained sequences of *Lichenostomus flavescens* (Accession Nos. AY488278 and AY488431), *Xanthomyza phrygia* (AY488314 and AY488471) and *Manorina melanophrys* (AY488282 and AY488435) from GenBank. These species are members of a larger honeyeater clade that includes *Meliphaga* (Driskell and Christidis, 2004).

2.2. Molecular methods

DNA was extracted from frozen and ethanol-preserved tissue using a standard phenol–chloroform extraction procedure as described in Gemmel and Akiyama (1996). For the core dataset the complete ND2 gene was amplified and sequenced as two overlapping fragments of 372 bp and 746 bp using the primers L5215 with H5578 (Hackett, 1996) and L5572 (CCA TAA AAC TAG GCC TAG TCC CAT T; this study) with H6315 (Kirchman et al., 2001), respectively. Remaining specimens were sequenced for the 372 bp fragment to verify taxonomic assignment and evaluate the presence of cryptic variation (see Appendix A). The FIB5 intron was amplified and sequenced using primers Fib5 and Fib6 following Driskell and Christidis (2004). PCR conditions for both gene regions were similar to those described by Driskell and Christidis (2004). Amplified fragments were purified using the GFX Gel Band and PCR Purification Kit (Amersham Bioscience Corp., Piscataway, NJ) and sequenced in both directions using the Dynamic ET Terminator Cycle Sequencing Kit (Amersham Bioscience Corp., Piscataway, NJ) in 10 μl reactions for 25 cycles under the following conditions: 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 1 min. Following purification on AutoSeq 96 plates (Amersham Bioscience Corp., Piscataway, NJ), sequencing products were separated on a MegaBACE 1000 capillary DNA sequencer using injection conditions of 3 kVA for 80 s and subjected to electrophoresis at 9 kVA for 100 min.

Sequences were aligned and edited using the program SEQUENCHER v.4.1.4 (Gene Codes Corp. Michigan, USA). The presence of large indels in FIB5 made automatic alignment problematic so sequences were aligned by eye. All informative sites in the final alignment were double-checked for accuracy. ND2 sequences were translated and checked for stop codons, anomalous substitution patterns and deviant base composition.

2.3. Phylogenetic analysis

Phylogenetic analysis of the concatenated dataset was performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods using the programs PAUP* (Swofford, 2002) and MRBAYES 3.1 (Ronquist and Huelsenbeck, 2005), respectively. For simplicity, indels were excised from the dataset and analysed separately as they have been shown to confer important phylogenetic information (Edwards et al., 2005; Prychitko and Moore, 2003). MODELTEST 3.06 (Posada and Crandall, 1998) was used to evaluate the best fit among 56 different evolutionary models for both data partitions and the concatenated dataset. The best fit to our concatenated dataset was a TVM + Γ model with a gamma-distributed rate variation ($\alpha = 0.2102$) and nucleotide frequencies of A = 0.3240, C = 0.3048, G = 0.1300, T = 0.2413. For ND2 a GTR + Γ model with a gamma-distributed rate variation ($\alpha = 0.2792$) showed the best fit with nucleotide frequencies of A = 0.3049, C = 0.3486, G = 0.1119, T = 0.2347, and for FIB5 a HKY85 + Γ model with a gamma-distributed rate variation ($\alpha = 0.1506$), a transition/transversion ratio of 1.9841 and nucleotide frequencies of A = 0.3296,

C = 0.2125, G = 0.1694 and T = 0.2885, outperformed all other models.

Heuristic ML and MP searches were run using the tree-bisection-reconnection method for tree-swapping starting from a neighbor-joining tree (ML) or stepwise addition using simple addition sequence (MP). Support for individual nodes was estimated through heuristic bootstrap resampling (100 and 1000 replicates, respectively). In our Bayesian searches, we ran four chains (one hot, three cold) for 1,000,000 generations, sampling trees every 100 generations. We ensured that our Bayesian runs achieved sufficient convergence by ascertaining that the average standard deviation of split frequencies between chains had reached below 0.01 at the end of the runs and by making sure that the potential scale reduction factor (PSRF) of each parameter stayed within $0.997 < \text{PSRF} < 1.003$. Plots of generation versus the log probabilities of observing actual data did not reveal any trends for the last 75% of generations. In order to set a conservative burn-in value, we therefore excluded the first 250,000 generations from the calculation of posterior probabilities.

In addition to bootstrap values and posterior probabilities, character-based measures of support were evaluated for all nodes using PAUP* in conjunction with the program TREEROT (Sorenson, 1999). These parameters included Bremer's (1988, 1994) support index (decay index or branch support; BS), partitioned branch support (PBS; Baker and DeSalle, 1997), hidden branch support (HBS) and partitioned hidden branch support (PHBS; Gatesy et al., 1999). These parsimony-based measures allow for the investigation into the relative contribution of each partition to selected nodes, and into the amount of hidden branch support concealed in separate analyses that unfolds when all partitions are combined (Gatesy et al., 1999). The utility of combining independent data partitions in molecular phylogenetic research has been strongly debated but the ad-hoc separation of datasets is also subject to shortcomings (Barrett et al., 1991; Chippindale and Wiens, 1994; DeSalle and Brower, 1997; Nixon and Carpenter, 1996; Siddall, 1997). Therefore, we conducted a combined analysis of our data and explored it in terms of relative character support and conflict contributed by the separate data partitions (Baker and DeSalle, 1997; Gatesy et al., 1999) rather than perform independent analyses.

2.4. Molecular clock calibration and estimates of divergence times

To assess the extent of agreement of a molecular clock to our data we generated ML trees with and without the enforcement of a molecular clock (Posada and Crandall, 2001) for the concatenated dataset and both data partitions. We checked whether a likelihood ratio test rejects the null hypothesis of a molecular clock, using $n-2$ degrees of freedom, where n is the number of taxa. Divergence times for ND2 sequences were estimated by applying a calibration factor of 0.561 ± 0.005 (95% confidence interval) to the Γ-corrected Kimura-2-parameter distances of our dataset. This correction factor allowed for direct application to our dataset of Fleischer et al.'s (1998) widely cited standard divergence rate of 1.6%/myr for Γ-corrected Kimura-2-parameter distances of cytochrome-b sequences in Hawaiian honeycreepers. The correction factor was determined empirically from regression of 1953 pairwise comparisons of Γ-corrected Kimura-2-parameter distances of cytochrome-b and ND2 sequences of 63 honeyeater species from the dataset of Driskell and Christidis (2004) (ND2: $\Gamma = 0.325$; cytb: $\Gamma = 0.221$). Our findings indicate a faster rate of ND2 evolution (2.8%/myr) in honeyeaters and contrast with a report of similar rates of ND2 and cytochrome-b evolution in dabbling ducks (tribe Anatini; Johnson and Sorenson, 1998).

3. Results

3.1. Molecular characterisation

The core ND2 dataset consisted of 1038 bp of sequence/individual, of which 392 (37.8%) were variable and 288 (27.7%) were potentially parsimony-informative. The length of the FIB5 fragment varied from 544 bp to 589 bp due to the occurrence of 6 indels which ranged in size from 1 to 47 bp. Of the 542 bp of aligned sequence (excluding indels), 66 (12.2%) were variable, with 44 (8.1%) being parsimony-informative. The concatenated dataset consisted of 1580 bp (with indels excised). All full length sequences from the core dataset have been submitted to GenBank with accession numbers listed in Appendix A. Additional sequences are available from JN on request.

3.2. Phylogenetic analyses

Bayesian analysis recovered a robust phylogenetic tree with high posterior probabilities ($\geqslant 88$) for most nodes (Fig. 2). Our ML analysis found one best tree (likelihood score 8515.8) in which 68% of all nodes exhibited bootstrap values higher than 80. The combined MP analysis found four most parsimonious trees of 1390 steps (consistency index: 0.462; retention index: 0.617). Both the ML and consensus MP trees were in agreement with the Bayesian topology except for their failure to recover node B which was only weakly supported in the Bayesian analysis.

Partition homogeneity tests (Farris et al., 1995) showed that the ND2 and FIB5 data partitions were incongruent ($p = 0.01$). Nevertheless, the total evidence approach yielded a phylogenetic tree that received strong support for almost all nodes (Table 1). ND2 revealed strong PBS for most terminal nodes (A, C, E, F, G, J, L and N) as well as the two basal nodes (D and P). The FIB5 data partition revealed high levels of PHBS in the combined analysis providing strong support for the more basal nodes (H, I, M, O and P). Six of the 18 nodes (A, C, I, L, P and Q) received positive support from both data partitions. Hidden branch support (Total PHBS; Table 1) increased support for many clades

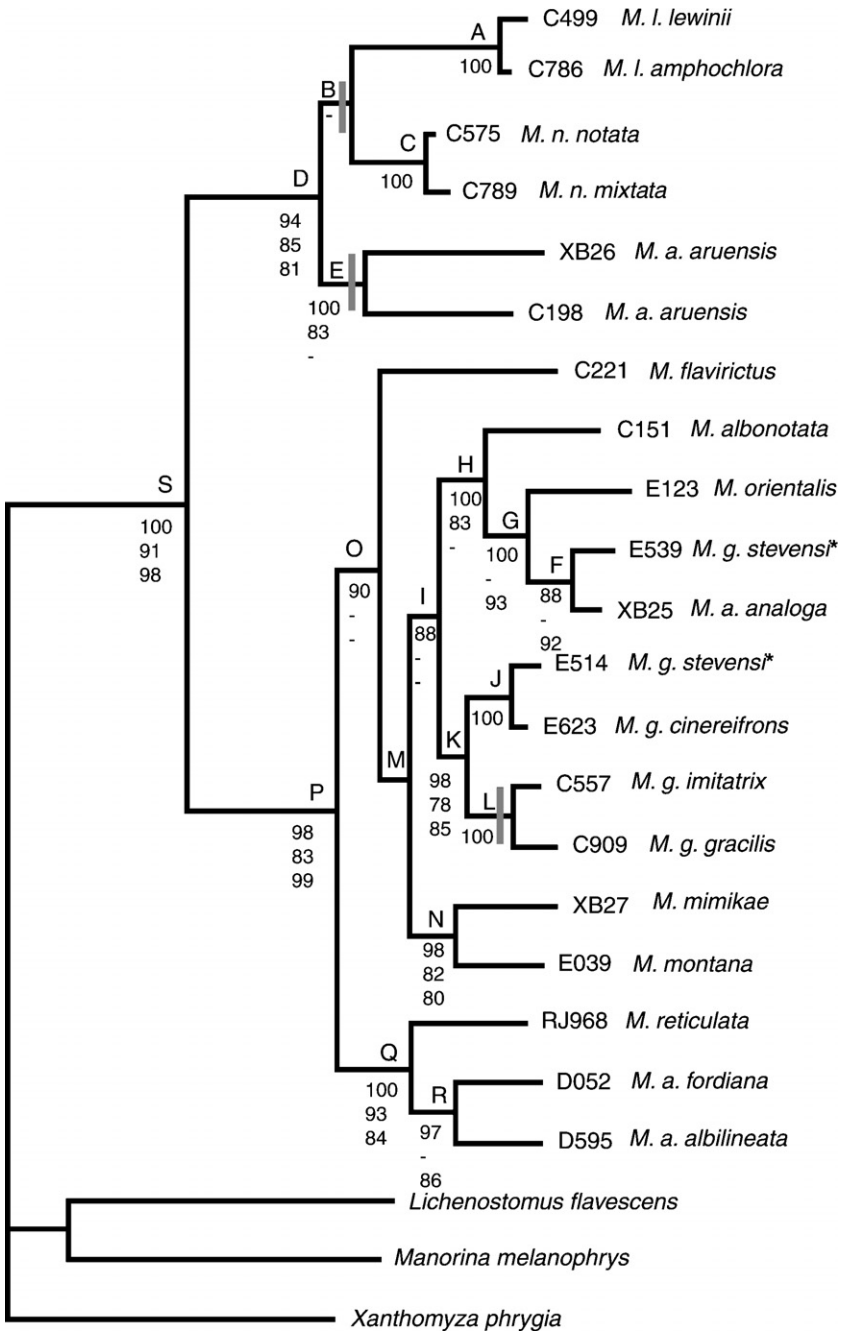

Fig. 2. Bayesian inference tree of the concatenated core dataset. Nodes are letter-coded for subsequent analyses. The three numbers at each node denote Bayesian posterior probabilities (uppermost), ML bootstrap (centre) and MP bootstrap (lowermost); "–" indicates weak support (<85 posterior probability; <75 bootstrap); a single value indicates identical support by all three methods. Grey crossbars on branches indicate the presence of phylogenetically informative indels; "∗" identifies taxa found to be polyphyletic.

including *M. lewinii–M. notata* (node B) which received low support in most other analyses. The ND2 and FIB5 data partitions showed distinct patterns of PBS and PHBS, reflecting their different molecular and evolutionary properties. Most notably, ND2 tended to provide strong PBS but little PHBS for terminal nodes reflecting a high signal-to-noise ratio at this level. In contrast, FIB5 tended to reveal high PHBS values especially for deeper nodes indicating a high degree of conflicting information (due to homoplasy or ancestral polymorphism) within this dataset.

The FIB5 data partition contained 6 indels three of which were phylogenetically informative (Fig. 2): (1) a 5 bp indel supporting node B; (2) a 1 bp indel supporting node E; (3) a 1 bp indel supporting node L. Two additional indels were present as autapomorphies, a 1 bp indel in C557 (*M. gracilis imitatrix*) and a 6 bp indel in E039 (*M. montana*). The remaining indel was highly polymorphic showing considerable length variation both within and between species and, along with the autapomorphies, was excluded from further analysis.

Table 1
Partitioned branch support (PBS) and partitioned hidden branch support (PHBS) of both data partitions for nodes labelled as in Fig. 2

Node	A		B		C		D		E		F		G		H		I	
	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS
ND2	30.5	-3.5	0	6	13	-2	19	6	6	6	14.5	0.5	7.5	0.5	-8.5	-0.5	3	2
FIB5	2.5	3.5	0	-3	3	0	-14	-4	-2	-2	-7.5	3.5	-2.5	1.5	8.5	9.5	-3	15
Total	33	0	0	3	16	-2	5	2	4	4	7	4	5	2	0	9	0	17
Node	J		K		L		M		N		O		P		Q		R	
	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS	PBS	PHBS
ND2	7.5	-8.5	0.5	-1.5	13	0	0	-3	11.5	4.5	-1.5	0.5	10	-3	3	-2	3.5	-3.5
FIB5	8.5	11.5	1.5	5.5	2	2	1	18	-7.5	-1.5	2.5	19.5	1	16	-1	4	-1.5	1.5
Total	16	3	2	4	15	2	1	15	4	3	1	20	11	13	2	2	2	-2

The totals of PBS and PHBS values for each node denote branch support (= Bremer's index) and hidden branch support, respectively. Positive values have a black background, negative values a white background, and zero values a grey background for ease of interpretation. Node B was not supported by a combined MP analysis and therefore received zero PBS for both data partitions.

All analyses resolved two well defined clades; a species-poor clade (*lewinii* group) comprising *M. lewinii*, *M. notata* and *M. aruensis*, and a larger clade (*analoga* group) containing the remaining species including *M. reticulata* from Timor, previously considered to be of uncertain affinities (Fig. 2).

3.3. Species limits and taxonomic nomenclature

Our phylogenetic analysis recovered deep branches within several species groups indicating the presence of cryptic speciation events. Uncorrected ND2 divergences for taxa assigned to *M. albilineata* (4.8%), *M. aruensis* (6.9–7.9%) and *M. gracilis* (3.4–4.2%) considerably exceed divergences observed between conspecifics (0.5–2.1%) of other taxa. In the case of *M. albilineata* and *M. aruensis*, divergences also exceed those observed between some sibling species pairs (*M. mimikae–M. montana*: 4.5–4.7%; *M. orientalis–M. analoga*: 4.4–4.6%). We also recovered specimens identified as *M. gracilis stevensi* as polyphyletic with the sequenced individuals, E514 and E539, clustering with *M. g. cinereifrons* and *M. analoga*, respectively. This pattern was also recovered in each of the single gene trees (data not shown).

Based on the phylogenetic relationships recovered herein we propose recognition of 16 species (Fig. 3, Appendix A) with the following taxonomic and distributional changes. This revised taxonomy is used in subsequent analyses and discussion.

(1) The allopatric forms of *M. albilineata* are elevated to species rank, *M. albilineata* (white-lined honeyeater) from Arnhem Land and *M. fordiana* (Kimberley honeyeater) from the Kimberley region of north-western Australia. The genetic and taxonomic distinctiveness of these species is consistent with results from a previous allozyme study (Christidis and Schodde, 1993).

(2) Within *M. gracilis* (graceful honeyeater) we retain the forms *gracilis* (C799 and C909; Cape York Peninsula, Australia and the Trans-Fly region, southwestern New Guinea) and *imitatrix* (C557; northeastern Australia). The southeastern New Guinean forms *stevensi* (E514; north coast) and *cinereifrons* (E623; south coast) are segregated from these at specific level. Both names have equal priority (Rand, 1936, p. 20) and, as first revisors, we select *M. cinereifrons* for this combination, with the recommended English name of elegant honeyeater.

(3) Specimens representing both *M. cinereifrons stevensi* (E514) and *M. analoga stevensi* (E539) have been collected from the northern watershed of southeastern New Guinea. The nominal form *stevensi* has proven difficult to place taxonomically, being assigned to either *M. gracilis* (Coates, 1990; Dickinson, 2003; Rand, 1936; Sibley and Monroe, 1990) or *M. analoga* (Salomonsen, 1967). We resolve this area of longstanding taxonomic confusion by providing evidence that *stevensi* comprises cryptic variants of both *M. cinereifrons* and *M. analoga*. In a subsequent paper we will determine to which complex the type of *stevensi* belongs and thus which names belong to these populations.

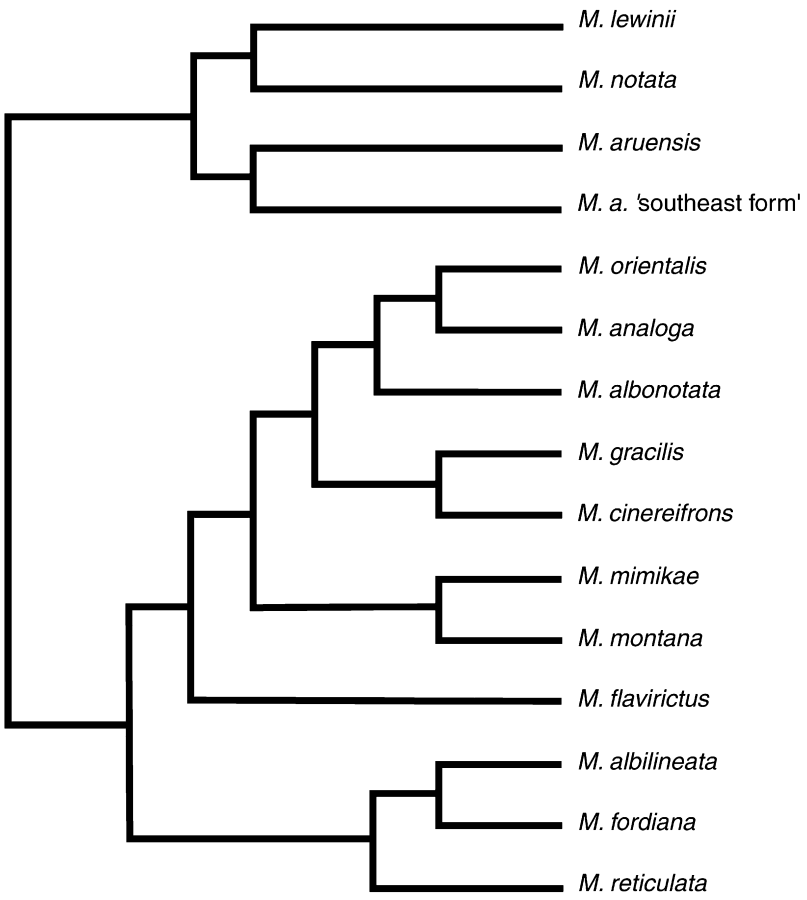

Fig. 3. Phylogram depicting relationships amongst the 16 *Meliphaga* species.

(4) Specimens from southeastern New Guinea currently assigned to *M. aruensis* (C198, E486) represent a distinct taxon at specific level. As the eastern form has not been formally named we provisionally refer to it as *M. aruensis* 'southeast form' pending further analysis, and retain *M. a. aruensis* for populations in southwest New Guinea and *M. a. sharpei* for populations to the north. A more detailed analysis of geographic variation within this complex is underway to fully resolve the affinities, status and distribution of the component forms.

3.4. Molecular clock and dating of divergence times

A likelihood ratio test including all specimens of the core dataset rejected the application of a molecular clock for the FIB5 partition and the concatenated dataset (FIB5: $\chi^2 = 49.16$, $p = 0.0018$; whole set: $\chi^2 = 48.28$, $p = 0.0023$). However, a ML tree that enforced a molecular clock on our ND2 partition was not a significantly worse fit than a non-clock tree ($\chi^2 = 31.67$, $p = 0.1353$), which suggests that our mitochondrial marker evolves at an approximately clock-like rate in *Meliphaga*. Application of a divergence rate of 2.8%/myr to Γ-corrected Kimura-2-parameter distances of the ND2 data ($\Gamma = 0.325$) resulted in estimated divergence times ranging from 6.3 ($\pm$0.9) to 0.2 million years ago (MYA). The earliest divergence corresponds to the separation of the two major clades (*lewinii* and *analoga* groups) with most subsequent speciation events estimated to have occurred during the Pliocene, between 5.3 and 1.8 MYA. Pleistocene differentiation events were restricted to subspecific diversification of populations within *M. lewinii*, *M. notata*, *M. analoga*, *M. gracilis* and *M. cinereifrons* (Fig. 3).

3.5. Modes of speciation

Our molecular phylogeny was used to investigate modes of speciation and ecological diversification in *Meliphaga*. The geographical distribution patterns of each species, along with their approximate spatial distributions (altitudinal and vertical), are plotted against their phylogenetic position in Figs. 4 and 5, respectively. Examination of the resulting topologies shows that recently evolved species occupy allopatric or parapatric ranges indicating the potential for both allopatric and ecological speciation mechanisms. Three nodes have given rise to sister lineages that replace each other geographically (*M. mimikae–M. montana*, *M. gracilis–M. cinereifrons* and *M. albilineata–M. fordiana*) whereas a single node has given rise to species which replace each other altitudinally (*M. analoga–M. orientalis*). We find no evidence of sympatric speciation

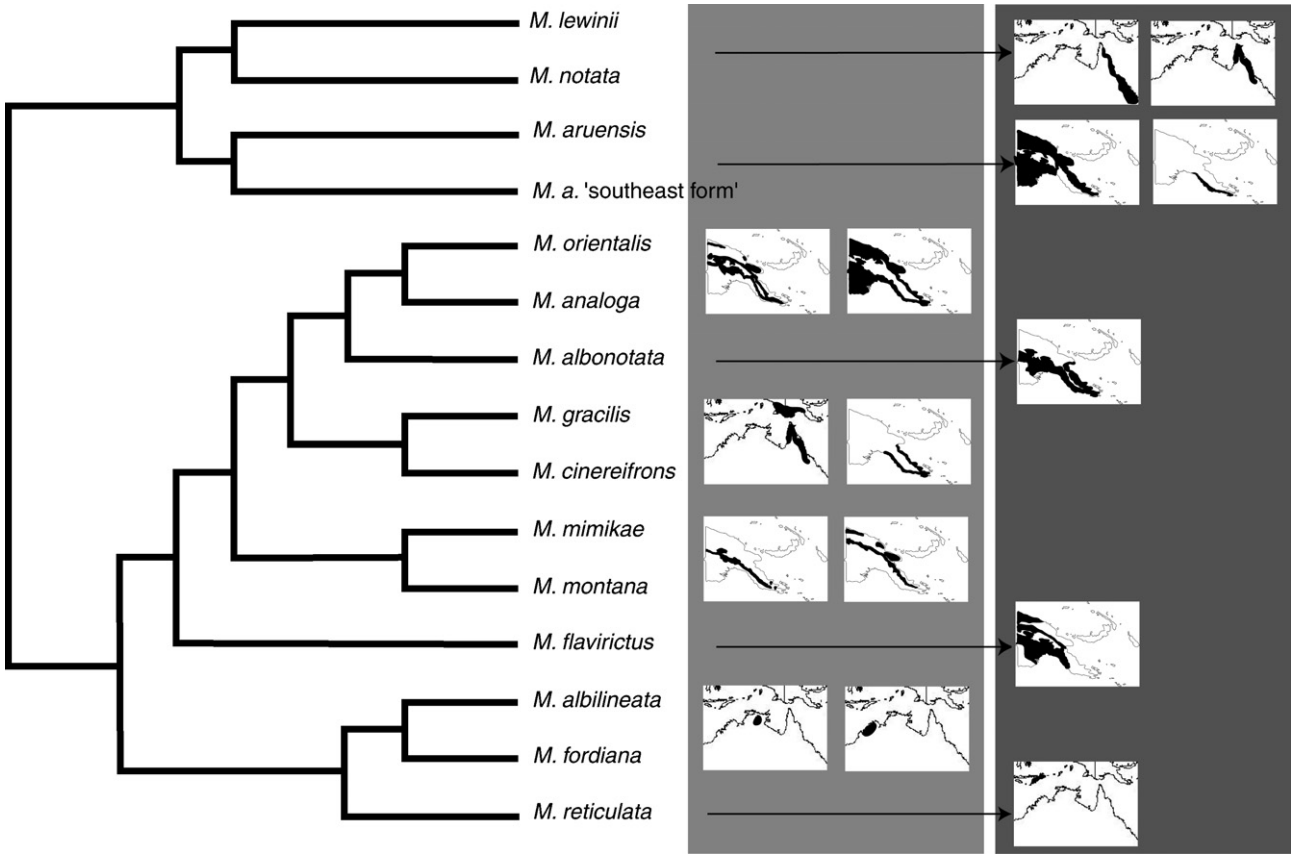

Fig. 4. Geographic distribution of *Meliphaga* species in relation to phylogeny. For simplicity, species distributions are shown for Papua New Guinea, northern Australia and Timor only and are adapted from Coates (1990), Coates et al. (1997) and Schodde and Mason (1999). For each species pair the geographic range of the upper taxon is depicted on the left and the lower taxon on the right. Distributions of recent sister lineages used to infer modes of speciation are indicated with a light grey background; older lineages are indicated by a dark grey background. Due to uncertainties concerning the distributional limits of *M. aruensis* and *M. aruensis* 'southeast form' the distributions shown are provisional only.

(co-distributed sister lineages) amongst recently evolved species and conclude that niche partitioning is not the primary speciation mechanism in *Meliphaga*. Due to the difficulties involved in reconstructing ancestral ranges for more ancient lineages we make no inference about their mode of speciation from current distributional patterns. Nevertheless we note that species which do occupy broadly concordant ranges are phylogenetically distant and in many cases are represented in different clades (e.g., *M. analoga* and *M. aruensis*) (Fig. 4).

4. Discussion

4.1. Molecular systematics and taxonomy of Meliphaga

The *Meliphaga* honeyeaters have presented one of the most difficult taxonomic problems amongst Australo-Papuan passerines. The genus currently comprises 13 species and 36 subspecies (Sibley and Monroe, 1990; Wolters, 1979). Although based on limited geographic sampling, the present study indicates that the genus *Meliphaga* contains previously undescribed taxonomic diversity and comprises at least 16 species; *M. vicina* and the 15 species identified in this study (Fig. 3, Appendix A). Phylogenetic analysis incorporating broader geographic sampling is necessary to determine the full extent of taxonomic diversity within this complex and the systematic affinities and distributional limits of each of the component forms.

Our phylogenetic hypothesis is in good agreement with independently derived allozyme data (Christidis and Schodde, 1993) in identifying a basal division of *Meliphaga* into two clades. The *lewinii* group comprises four species; *M. lewinii* and *M. notata* from Australia along with *M. aruensis* and the newly identified *M. aruensis* 'southeast form' from New Guinea. The *analoga* group is a larger, predominantly New Guinean radiation, comprising the remaining 12 species. Although not sampled, we follow Christidis and Schodde (1993) in including *M. vicina* in this group because of its apparent affinities with either *M. analoga* or *M. gracilis* (Salomonsen, 1967; Sibley and Monroe, 1990) with which it may be conspecific. The *analoga* group also contains the extra-limital species *M. reticulata* from Timor which is closely aligned with *M. albilineata* and *M. fordiana* from northern Australia. Although traditionally retained within *Meliphaga* (Coates et al., 1997; Schodde, 1975; Sibley and Monroe, 1990; Wolters, 1979), *M. reticulata* has also been placed within the genus *Lichenostomus* (Dickinson, 2003) with possible affinities to the ornate honeyeater

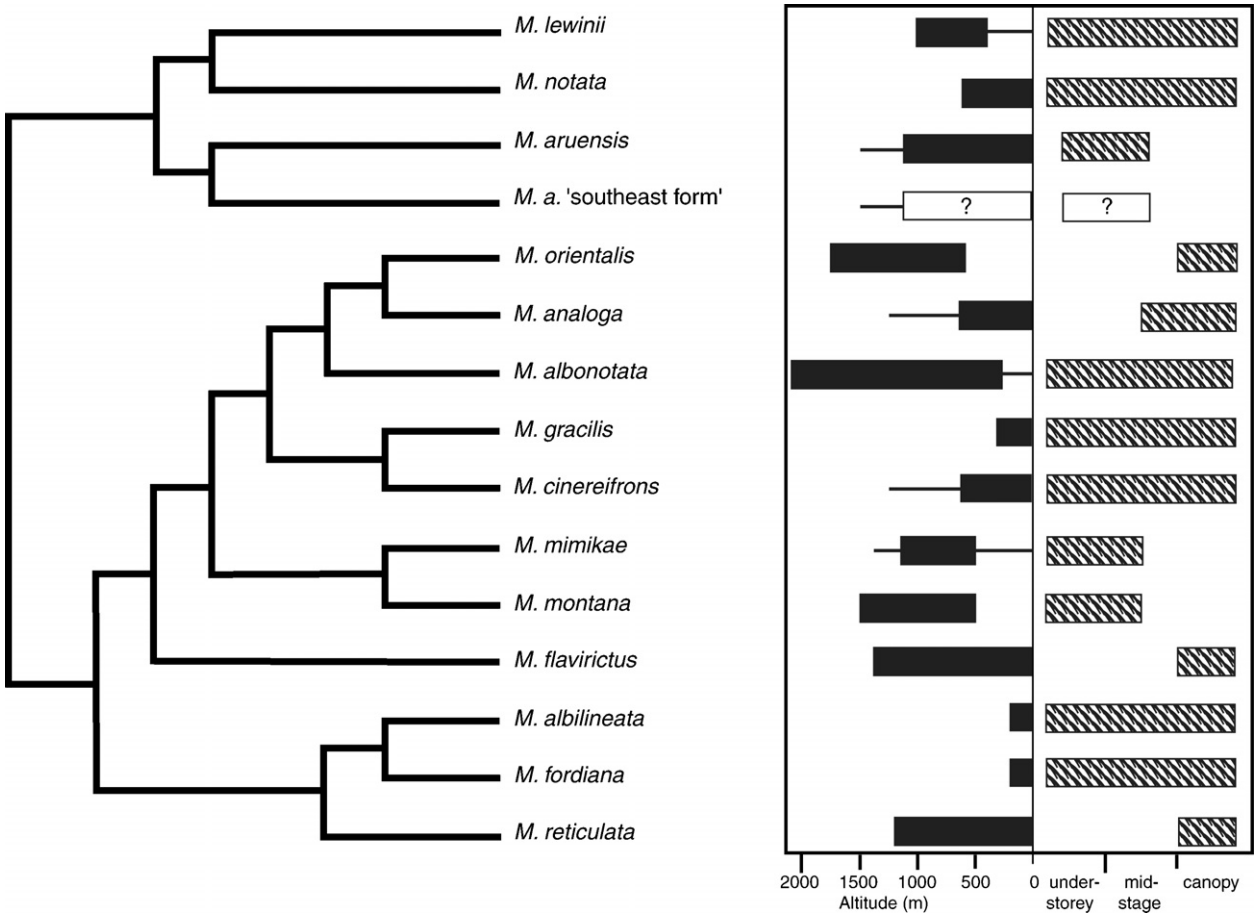

Fig. 5. Spatial distributions of *Meliphaga* species indicating altitudinal range in metres (left graph) and vertical zonation within the habitat (right graph) following information in Coates (1990), Coates et al. (1997) and Higgins et al. (2001). For altitudinal range, rectangular boxes indicate the core range with horizontal bars indicating altitudinal limits reported for some local populations. The spatial characteristics of the newly identified *M. aruensis* 'southeast form' are tentatively shown as being similar to *M. aruensis*.

(*L. ornatus*; White and Bruce, 1986). Despite acknowledged plumage similarities between *M. reticulata* and *M. albilineata–M. fordiana*, a close relationship between them has been dismissed (White and Bruce, 1986). Although we find strong molecular support for the presence of two clades in *Meliphaga,* corroborating morphological (plumage) characters appear to be lacking.

4.2. Historical biogeography

Australia is recognised as the centre of diversity for the Meliphagidae with 72 of the 169 species of honeyeater occurring there (Dickinson, 2003), as well as Australian endemic *Acanthorhynchus* being the basal lineage (Driskell and Christidis, 2004). Although *Meliphaga* is unusual in reaching its highest species diversity in New Guinea our phylogeny is unable to distinguish between Australia and New Guinea as alternative centres of origin for the genus as both hypotheses provide equally parsimonious solutions. Nevertheless, both major clades (*lewinii* and *analoga* groups) are deeply divided into Australian and New Guinean lineages, possibly initiated by the formation of the Arafura Sea.

Our molecular clock estimates suggest that the radiation of *Meliphaga* in New Guinea occurred during the Pliocene (5.3–1.8 MYA) with the most recent trans-Torresian interchange involving an early Pleistocene (1.2–1.5 MYA) reinvasion of Australia by the open-habitat species *M. gracilis*. Timor was likely colonised from Australia ~2.8 MYA. Within the limitations of molecular date estimates, most events predate the formation of land connections under the model proposed by Flannery (1995) and contribute to a growing body of evidence supporting Miocene-Pliocene faunal interchange between Australia and New Guinea. Although there is limited geological evidence for the presence of land connections between Australia and New Guinea (or Timor) during this period, Hall (2002) emphasises the difficulties of making such inferences. Consequently, we refrain from attributing these periods of interchange to long distance open water dispersal. Both Timor and New Guinea occur at the edge of the Australian continental shelf, large areas of which became exposed during the Pleistocene glacial episodes as a result of intermittently falling sealevels (Jones and Torgersen, 1988). Whether conditions favoured the formation of partial land connections or stepping stones during earlier periods must

remain open for speculation in light of accumulating biogeographic and geological evidence. Indeed, the most recent trans-Torresian exchange in *Meliphaga* involves *M. gracilis*, one of the few open habitat specialists. The majority of avian species with trans-Torresian distributions are represented in, or restricted to, the Trans-Fly region of New Guinea and occupy similar open habitats (e.g., tidal mangroves, swamp forest, savannah, secondary growth and forest edge). They include several honeyeaters (rufous-banded *Conopophila albogularis*, white-throated *Melithreptus albogularis*, blue-faced *Entomyzon cyanotis* and brown *Lichmera indistincta*), olive-backed and yellow orioles (*Oriolus sagittatus* and *O. flavocinctus*), the Australian magpie *Gymnorhina tibicen*, and fawn-breasted bowerbird *Chlamydera serviniventris*. These marginal or peripheral habitats are the ones most likely to develop on recently emerged land masses and would predate the formation of closed forest systems necessary for dispersal of other species. Corroboration of the timing of other trans-Torresian avian interchanges would shed enormous light on the potential timing of any such land connections.

4.3. Ecological diversification and modes of speciation

As predicted by Diamond (1972), the evolution of spatial sorting mechanisms can be used to explain the distribution of *Meliphaga* species. Combining information from geographical, altitudinal and vertical distributions we find that nearly all *Meliphaga* species can be partitioned using these parameters alone. In all cases, closely related species occupy disjunct distributions and replace each other geographically or altitudinally thereby minimising competition between ecologically similar forms. In contrast, co-distributed lineages tend to be phylogenetically distant and coexist by segregating vertically within the habitat (e.g., *M. aruensis*, *M. analoga* and *M. flavirictus*). Exceptions are *M. albonotata*, *M. gracilis* and *M. cinereifrons* which minimise overlap with other species through horizontal segregation, habitat differentiation and/or a restricted altitudinal range. For instance, *M. albonotata* is restricted to forest edge and rarely ventures into the forest interior (Coates, 1990; Diamond, 1972), thus avoiding contact with *M. aruensis*, *M. analoga* and *M. flavirictus* in the lowlands, and *M. mimikae*, *M. montana* and *M. orientalis* in the highlands.

The predominantly allopatric distributions observed amongst recently evolved *Meliphaga* species suggest that most speciation events are initiated through spatial isolating mechanisms involving vicariance of single habitats (Mayr, 1963). This includes potential mountain uplift isolating northern *M. montana* and southern *M. mimikae* in New Guinea, flooding of the Arafura basin (or open water dispersal) isolating Australian *M. gracilis* and New Guinean *M. cinereifrons*, and aridification isolating *M. albilineata* and *M. fordiana* in northern Australia. In New Guinea, the pattern of altitudinal replacement observed between *M. analoga* and *M. orientalis* conforms to an explicit prediction of the ecological model of speciation (Moritz et al., 2000) in which sister species occupy distinct but adjacent (parapatric) habitats. These species replace each other altitudinally at ~500 m along an environmental gradient involving changes in vegetation type, temperature regimes, rainfall patterns and cloud cover (McAlpine et al., 1983; Paijmans, 1976). Most notably, areas above 500 m experience lower daily temperature minima, almost constant cloud cover and support a structurally and floristically different forest type being denser and more homogeneous than forests of the hills and lowlands below.

Diamond (1972) predicted altitudinal replacement to be a dominant mechanism of speciation in the New Guinean avifauna and this study provides the first empirical support for the hypothesis. Nevertheless, additional studies investigating patterns of morphological divergence and geneflow across these altitudinal gradients will be necessary to verify if diversifying selection is operating to promote speciation as predicted by the ecological gradient model. Alternatively, altitudinal replacement as observed in *M. analoga–M. orientalis* may be the result of geographic isolation in altitudinally segregated habitats with subsequent range expansions bringing the species into secondary contact. Under this model, evidence of population expansion would be predicted and such signatures can be detected using molecular population data (Rogers and Harpending, 1992).

Although the turbulent Neogene paleoclimatic and tectonic history of New Guinea might be expected to lead to novel speciation mechanisms with respect to their Australian counterparts we find that similar processes have led to the diversification of *Meliphaga* in the two regions. Of the five species that occur in Australia two, *M. albilineata* and *M. fordiana*, are closely related sister species that replace each other geographically. The remaining three, *M. lewinii*, *M. notata* and *M. gracilis*, are all phylogenetically divergent and occupy similar ranges within northeast Australia. While the more broadly distributed *M. lewinii* tends to segregate altitudinally in this region, *M. notata* (*lewinii* clade) and *M. gracilis* (*analoga* clade) co-occur showing slight differences in patterns of microhabitat selection (Higgins et al., 2001). These findings suggest phylogenetic constraints on modes of speciation and cautions against using patterns of diversification in one taxonomic group to infer evolutionary processes for a regional fauna. However, we do find evidence of differential rates of diversification between the two *Meliphaga* clades with the *analoga* group which comprises 12 of the 16 species, radiating more recently into a wider range of ecological, elevational and geographic zones within New Guinea. Our study suggests that comparative analysis of patterns of diversification in other Australo-Papuan avifaunal assemblages, including detailed intraspecific phylogeographic studies, are required to fully unravel the likely complex history of speciation in this region. Confirmation of altitudinal replacement in the New Guinean avifauna further suggests that this region will be a productive area for research into the role of ecological speciation mechanisms in the diversification of tropical faunas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.05.032.

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