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The influence of space and time on the genetic architecture of rail species (Aves: Rallidae)

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Evolutionary Ecology at Massey University, Palmerston North, New Zealand

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2014

Abstract

The main subject of this PhD research is the study of the underlying processes of evolutionary changes that lead to biological diversity. Such processes include those operating within and between populations (population divergence), as well as those operating among species (speciation), above the species level (e.g. genera and families) and the mechanisms that promote these divisions. Fundamental to these processes are the effects of genetic, demographic, geographical, ecological, behavioural and environmental factors on diversification. Rails (Aves: Rallidae) are used as an example to address central questions related to how these biological entities originated, when was that biological diversity generated, and why this biodiversity is distributed as it is. This thesis has been divided into four main chapters/papers for convenience to achieve this aim. In the first chapter, complete mitochondrial genomes and fossil data are used to provide a likely estimated time of rail ecology. I estimated that the origin and diversification of crown group Rallidae was during the Eocene about 40.5 (49–33) Mya with evidence of intrafamiliar diversification from Late Eocene to Miocene time. This time is much older than currently available fossils assigned to Rallidae, but more direct evidence of fossils with reasonable taxonomy are likely to emerge. This estimated time implies that rail diversity originated deep in the avian tree supporting an inference of deep ancestry of terrestrial/walking habits among Neoaves. In addition, in the second chapter I used neutral molecular data (nuclear and mitochondrial gene fragments) to reveal the degree of historical biogeographic signal and net diversification in the current lineage distribution using the most complete species-level hypothesis for ralloids. This comprehensive intrafamiliar molecular phylogeny allowed to infer spatial and ecological diversification in Rallidae associated with morphological innovation (frontal shield, body size and flightlessness) and the global retention of diversity in several lineages caused by dispersal, adaptation and exploitation of ecological opportunities. In the third and fourth chapters I explored historical patterns of diversification in a biogeographic context (in spatial and temporal scales) within a clade (*Porphyrio* but focused on the type species *Porphyrio porphyrio*) and a highly polymorphic species (*Gallirallus philippensis*). In the third chapter, a dated phylogeny and the tools of population genetics were used to gain insights into the congeneric relationships, diversification, and the history of expansion of one of the most peculiar clades within Rallidae. I found that the *Porphyrio* clade arose during the Mid-Miocene, apparently in Africa, with a single Long-Distance Dispersal (LDD) event occurring into the Americas and several other LDD events to the North-East around 10 Mya. *Porphyrio porphyrio* was not found to be a natural group with *P. melanotus* appearing in Australasia during the Pleistocene (600 kya). Dispersal, isolation, adaptation and selective pressure accounted for most of the variation found within this clade. On a finer scale, the fourth chapter explored genetic changes

within populations of the supertramp and great speciator *Gallirallus philippensis* using a mitochondrial DNA marker to recognise the genetic changes caused by founder events and provide important insights into the microevolutionary processes that drove the early stages of diversification. This study found that abrupt genetic changes of founder events are related to dispersal, colonisation, range expansion, gene flow, isolation and strong selection forces. The consequences of such processes for speciation and how they affected the population demography and evolutionary history of *Gallirallus philippensis* in the south western Pacific are discussed. These independent but linked studies within this thesis yield important clues to the evolutionary history that has shaped the diversity of rails. This research contributes to our understanding of Tertiary vertebrate evolution and establishes a bridge between macro– and micro–evolution.

Key words: Aves, biogeography, colonisation, dispersal, diversification, DNA, ecology, evolution, extinction, gene flow, isolation, phylogenetics, population genetics, Rallidae, speciation.

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At this stage is pleasant to look back and recall the names of those who in one way or another have helped me to complete this project.

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Thank you to New Zealand (Aotearoa -the land of the white cloud-): this beautiful and peaceful country where my dreams come true every day.

Contributions of others to this thesis: So far three chapters from this thesis have been submitted for publication in peer-review journals and the last chapter is in preparation for submission. I carried out all the laboratory work, data analyses and writing of drafts. Co-authors for all the manuscripts provided various levels of advice and discussion on drafts. Some data used in Chapter 4 were provided by Leo Joseph at the Australian National Wildlife Collection (ANWC), Greg Adcock at the University of Canberra (UC), and Faye Lux and Julian Reid at the Australian National University (ANU).

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Introduction

The purpose of this research is to contribute to a growing body of knowledge in evolutionary ecology through reasoning, question/hypothesis evaluation, and analyses of population dynamics and phylogenetic approaches. This PhD study is divided into four chapters to achieve an integrative structure and to explain evolution of organismal diversity and its geographical distribution. The reader should find in the following chapters an opportunity to understand the evolutionary history of one of the most remarkable groups of vertebrates, the rail birds (Aves: Rallidae), from the fundamental Darwinian evolutionary and Mendelian population genetic theories and Mayrian view of speciation. These studies have used basic scientific concepts along with new ideas, conceptual frameworks, theoretical approaches, molecular data, and bioinformatic tools to answer how, when and why rail diversity arose, diversified and spread.

Evolutionary ecology research has exploded over the last decades and continues to grow. In particular, new genomic tools allow new types of questions to be addressed and classical questions to be reexamined with more rigour and often supported by the findings of additional fossilized organisms. In this thesis I apply phylogenetics and population genetic analyses to unravel particular aspects of the evolutionary history of rails. In this pursuit I examine broader issues, such as speciation; evolution of ecological, morphological, and behavioural traits; relevance of population-level processes to phylogeny at the species-level and above; and potential causes of the geographical and temporal variation in diversification.

Two major goals of this thesis are to identify the patterns of diversification of rails and to infer geographical and ecological processes driven evolutionary differentiation through time. To achieve these goals I used (1) broad taxonomic and population sampling, (2) accurate estimation of phylogenetic relationships and population parameters, (3) substantial molecular data, and (4) fossils for which there are realible data to date processes of evolution and calibrate molecular clocks. In terms of the significance for interpretating the history of life this study provides a likely estimated time of rail ecology with integration of spatio-temporal patterns. The pattern of diversification, timing of the origin of this bird

family and its clades and the mechanisms operating at a microevolutionary level allows an interpretation of the phenotypic, ecological and geographical processes of evolution during the Tertiary and places this research more firmly in a broader avian evolutionary context when compared with other studies in different spatial (global/local) and temporal (deep/shallow radiations) scales. Therefore, this study has a high potential impact on current avian evolutionary work and adds important topics to the literature on evolutionary ecology.

Prior to this thesis, most of the knowledge of the evolution, taxonomy, systematics and biogeography of Rallidae was synthesised in the remarkable work of Ripley (1977); although most of the taxonomic treatment and classification in rails have been based on Olson (1973). The knowledge of this family was (and still is) "little and obscure" (Ripley 1977) but over the last few decades there has been increasing interest in recognising its diversity, genetic variation within species, population structure processes, intrafamiliar and interordinal evolutionary relationships, and patterns of distribution. Overall, the family Rallidae comprises approximately 85% of Gruiformes diversity and 1.3% of the diversity of extant birds. This family is composed of some 135-143 species within 33-40 genera (Taylor 1998; Houde 2009; Clements et al. 2012; Lepage 2013). Rallidae is divided into two subfamilies, Himantornithinae (with the single species *Himantornis haematopus*) and Rallinae, and about 39% of the genera are monotypic (Figure 1). Rails are problematic for biogeographic analysis because of the difficulties in defining relationships by the currently inadequate taxonomy, their widespread distribution, ability to disperse and colonize with a propensity to evolve flightless species, apparent poor flight ability and broad morphological diversity.

The ancestral ecology and geographic origins of modern rails is almost entirely unknown but most of the putatively basal species, as well as several distinctive genera, inhabit forests of the Old World tropics (Olson 1973). Fewer genera are found in the New World, and most of these are interpreted as being derived from an Old World stem (Olson 1973). Some genera (e.g. *Rallus* and *Fulica*) appear to have specialized and radiated in the Americas before reinvading the Old World (Olson 1973). Four genera as currently treated (*Porzana,*

Porphyrio, Gallinula, and *Fulica*) have a worldwide distribution (Figure 2). *Porzana*, as currently recognized, is the most speciose genus in the family (11.9% of rails are currently classified as *Porzana*), followed by *Gallirallus* (11.2%), *Fulica* (7.7%), and *Gallinula, Laterallus, Rallus, Amaurornis* and *Sarothrura* (6.3%). Most of the diversity of *Porzana* and *Gallirallus* is found in Asia and Oceania (Figure 2) along with three endemic genera in each of these continents (*Aramidopsis, Habroptila* and *Gallicrex* are only found in Asia and *Nesoclopeus, Eulabeornis* and *Megacrex* only in Oceania). Of these, all except *Nesoclopeus* are monotypic. Africa has 16 endemic species in seven endemic genera (including *Atlantisia* in the South Atlantic islands), 54 species occur in America, including 27 in eight endemic genera and six of the nine *Rallus* species, while only nine species occur naturally in Europe.

The fossil record for Rallidae is sparse and reveals little information about the origin, ecology and behaviour of the group. Adequate diagnostic material of fossil rail genera comes from the Upper Oligocene and Lower Miocene, 20–30 Mya, and by this time the birds had attained osteology similar to that of modern rails (Cracraft 1973; Olson 1977; Mayr 2006). Most of the fossil rails from Pliocene and Pleistocene in continental deposits have been assigned to modern genera. DNA-DNA hybridization studies suggested that rails may have diverged from the other gruiform groups as long as 86 million years ago (Mya), in the Upper Middle Cretaceous (Sibley and Ahlquist 1990), but analysis of short mitochondrial and nuclear sequence (Figure 3) suggests Mezosoic-Cenozoic boundary divergence of Grues into a rail-like lineage 66 Mya, the split of Ralloidea about 45 Mya (Houde 2009), and the origin of Rallidae clade around 20 Mya (Ericson et al. 2006; Brown et al. 2007; Fain et al. 2007; Houde 2009).

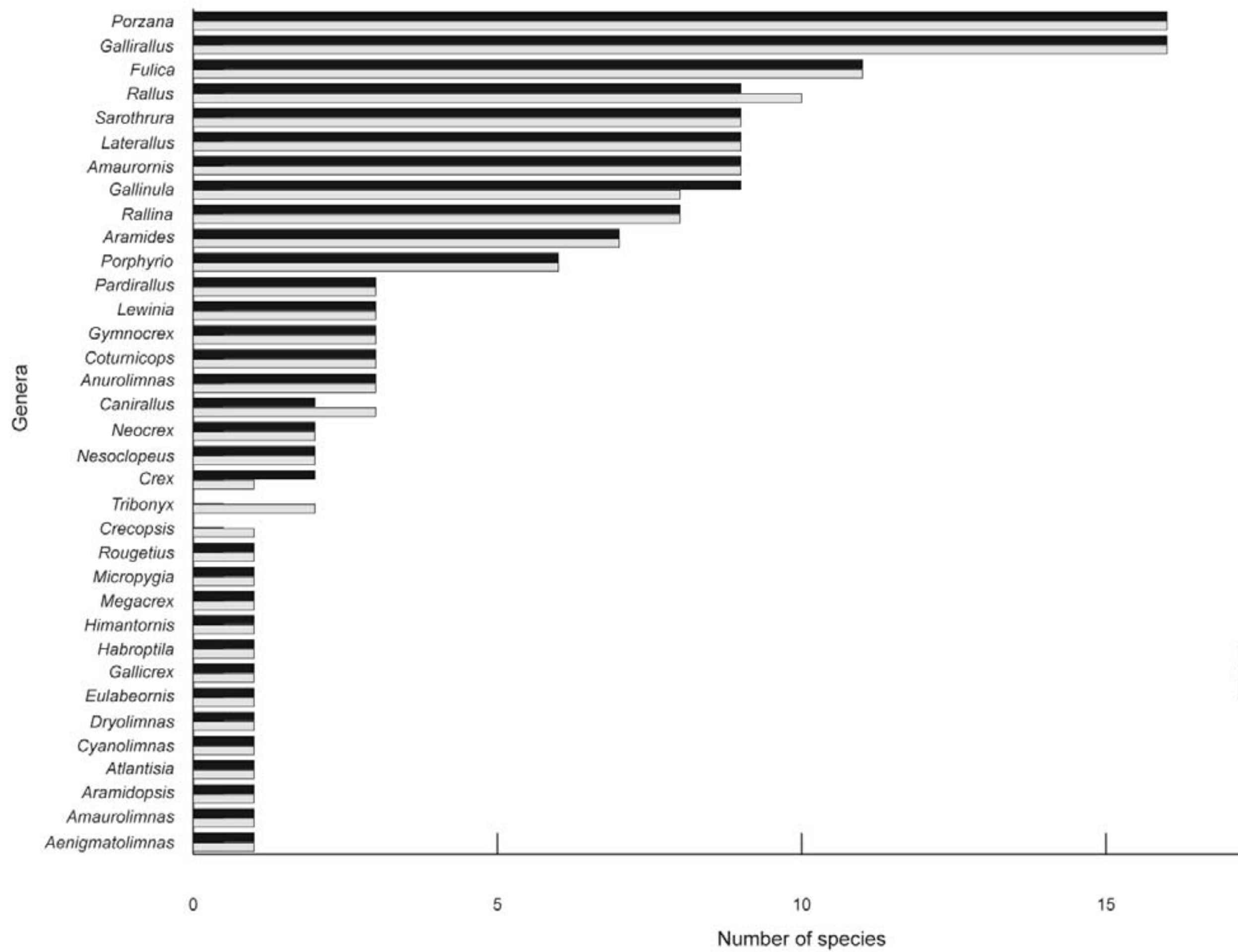
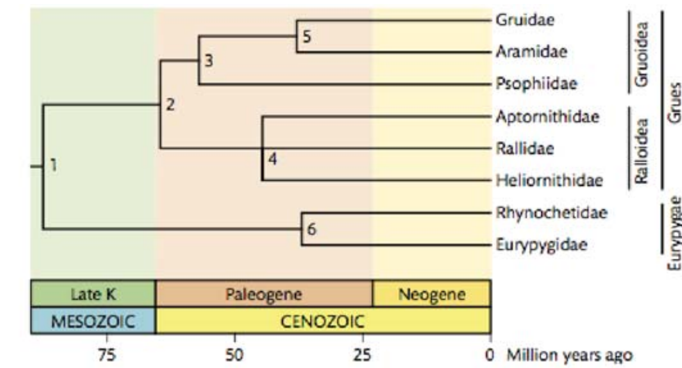


Figure 1. Summary of extant and recently extinct taxonomic diversity in the most up to date treatments of Rallidae following Ols
differences in the number of genera and species among these classifications because new taxa have been genetically verified e.g. *R*
and *Canirallus beankaensis* (see Groenenberg et al. 2008; Tavares et al. 2010; Goodman et al. 2011) and the separate treatment of
placed in the genus *Crecopsis*) and two species of *Gallinula* (*G. ventralis* and *G. mortierii* placed in *Tribonyx*) by Clements (201
can be found in Sharpe (1894), Peters (1934), Berndt and Meise (1960), Fisher and Peterson (1964) and Ripley (1977). For a sur
rallids see Taylor (1998) and Livezey (1998).

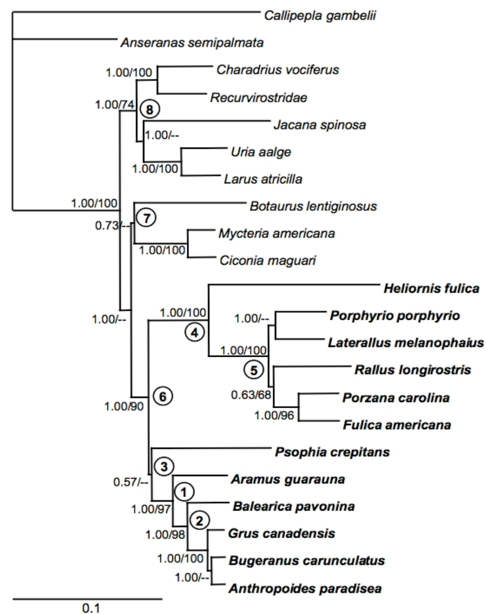
Figure 2. Proportional diversity of genera in Rallidae by geographic regions (Continents). Genera endemic to each continent are i
common to more than one region are indicated by identical colour in each pie chart.

a)



Timetree		Estimates						
Node	Time	Ref. (3)		Ref. (15)(a)	Ref. (15)(b)	Ref. (31)	
		Time	CI	Time	Time	Time	CI
1	87.4	-	-	74.5	88.2	99.5	123–83
2	64.5	73.2	107–50	45.1	69.0	70.5	89–58
3	56.9	66.4	98–45	32.0	63.8	65.4	83–53
4	44.5	42.6	66–27	45.1	46.9	43.2	57–33
5	37.8	48.5	74–31	17.4	39.4	45.9	61–35
6	36.8	-	-	31.4	38.1	41	56–30

b)



Node	Clade	Divergence time (MY)	95% credible interval
1	Aramidae–Gruidae	48.5	(31.3,73.8)
2	Gruinae–Balearicinae	31.4	(19.1,49.8)
3	Psophiidae	66.4	(44.8,97.8)
4	Heliornithidae–Rallidae	42.6	(27.1,65.5)
5	Basal Rallidae	21.8	(12.9,35.6)
6	Ralloidea–Gruoidea	73.2	(50.0,107)
7	Ardeidae–Ciconiidae	80.7	(55.9,117)
8	Basal Charadriiformes	74.3	(54.6,105)

Figure 3. Chronograms with tables summarizing confidence intervals of estimated times of divergence for (a) Ralloidea based on mean estimates of nuclear genes from studies considered by Houde (2009), and (b) Rallidae crown group (node 5) using multilocus analysis of Fain et al. (2007).

Due to the advancement and use of molecular markers it has been possible to expand our knowledge in taxonomy and systematics (morphological data supported, especially in the case of fossils), and biogeography and evolution of this group of birds (e.g. Trewick 1997; Slikas et al. 2002; Fain et al. 2007; Groenenberg et al. 2008; Kirchman 2009; Ozaki et al. 2010; Kirchman 2012; Ruan et al. 2012) but as astonishing as it seems, many important questions remain unanswered. Classification schemes and phylogenetic hypotheses of Rallidae have mostly dealt with subsections of the extant and extinct rail fauna. So far no molecular study has had sufficient breadth of taxa to tackle thoroughly the core problems (Figure 4). However, estimations of lineage cladogenesis are sensitive to taxon sampling, noncomparability of lineages and inaccurate phylogenetic relationships. Taxonomic classification also influences inferences about the rate and pattern of ecological diversification. Unravelling the deeper evolutionary and ecological histories of this group of birds requires extensive sampling of the several phylogenetic clades that span local and continental regions, even though parameters about timing, distribution and ecology are less constrained. Linking deep phylogenetics with analysis of speciation patterns and processes is hugely challenging, primarily because increasing phylogenetic time is accompanied by increasing uncertainty about what taxa have existed. Extinction, resulting in incomplete phylogenies, is apt to give false or at least uncertain impressions of diversification rates (Crisp and Cook 2009). This is reflected, for instance, in studies showing poor phylogenetic resolution at deeper nodes mostly because they have been based on analyses that used few species to represent families (e.g. Ericson et al. 2006; Brown et al. 2007; Pacheco et al. 2011). Analyses with additional lineage sampling would provide insights into more realistic biological diversification processes, revealing the evolutionary history, relationships, rates and patterns of diversification, and the degree of historical biogeographic signal and net diversification retained in the current lineage distribution.

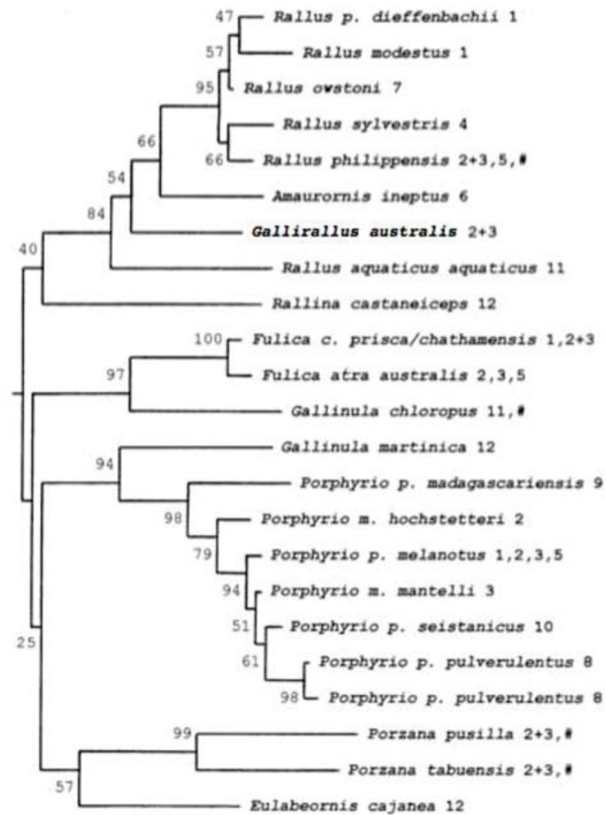
Inferring the spatio-temporal pattern of diversification in ralloids is challenging due to their capacity to fly and disperse over long distances. Dispersal and colonisation are important drivers in diversification of rails (Figure 5) but this is a paradox because they have a terrestrial lifestyle with flight mostly associated with predator avoidance (Trewick 2011). Colonisation events from extralimital dispersal within a rapidly multiplying supertramp

lineage (e.g. *Porphyrio*, *Gallirallus, Porzana, Gallinula*) can result in speciation events of flightless species and/or numerous phenotypically distinct variants, where mobility is not integral to predator avoidance, i.e. isolated oceanic islands without mammal predators (McNab 1994; McNab and Ellis 2006; Steadman 2006). Natural selection and the rate, constancy and directionality of gene flow played a key role in the tendency of rails to evolve flightless species. However, space and time are central aspects within the biodiversity composition. The importance of geographic settings and age of clades in shaping biodiversity patterns are illustrated throughout the different chapters of this thesis.

The reader must be aware that individual chapters were written in the form of manuscripts that have been submitted for publication or prepared for submission in peer-review journals, therefore some repetition might be found, particularly within the Material and Methods sections. I have not made changes to the manuscripts except in their format for this thesis (e.g. embedding tables and figures in the main text) to retain the structure of the papers. Below, I present a short overview of each chapter to summarise the content of this thesis.

Chapter 1 is entitled "Eocene diversification of crown group rails (Aves: Gruiformes: Rallidae)". In this chapter, the time and pattern of diversification of the family Rallidae is investigated using fossil calibrations, molecular clocks and complete mitochondrial genomes in a Bayesian framework. The evolution of this modern bird family in a spatial and temporal context is examined using available fossils. The age of modern bird families and the ecologies that they characterize create a platform for further biogeographic and evolutionary studies. I found that the estimated time of origin of Rallidae was in the Eocene about 40.5 (49–33) Mya with evidence of intrafamiliar diversification from Late Eocene to Miocene time. This timing is older than previously suggested for the crown group Rallidae (based on confident fossils assigned to Rallidae); however, robust fossil calibrations, broad taxonomic sampling and substantial sequence data give it credence. Fossils of Eocene age tentatively assigned to Rallidae will further support these findings. Compared to the age of origin of other bird lineages, the rail clade is old. This result implies that rail ecology is relatively old too and supports an inference of deep ancestry of ground-dwelling habits among Aves.

a)



b)

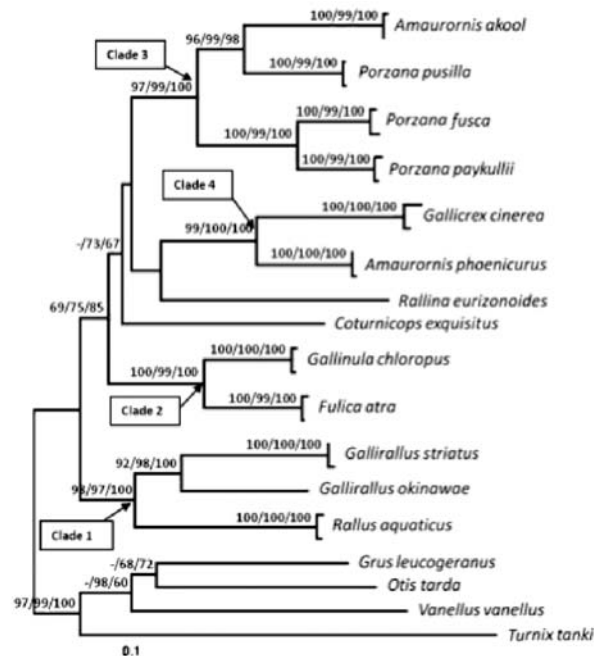


Figure 4. Summary of the generic and species relationships among rails in previous studies. a) 12S RNA mtDNA sequences study of Trewick (1997), and b) three mitochondrial genes (COII, ATPase 8 and cyt*b*) study of Slikas et al. (2002). Other recent studies are Kirchman (2012) and Ruan et al. (2012).

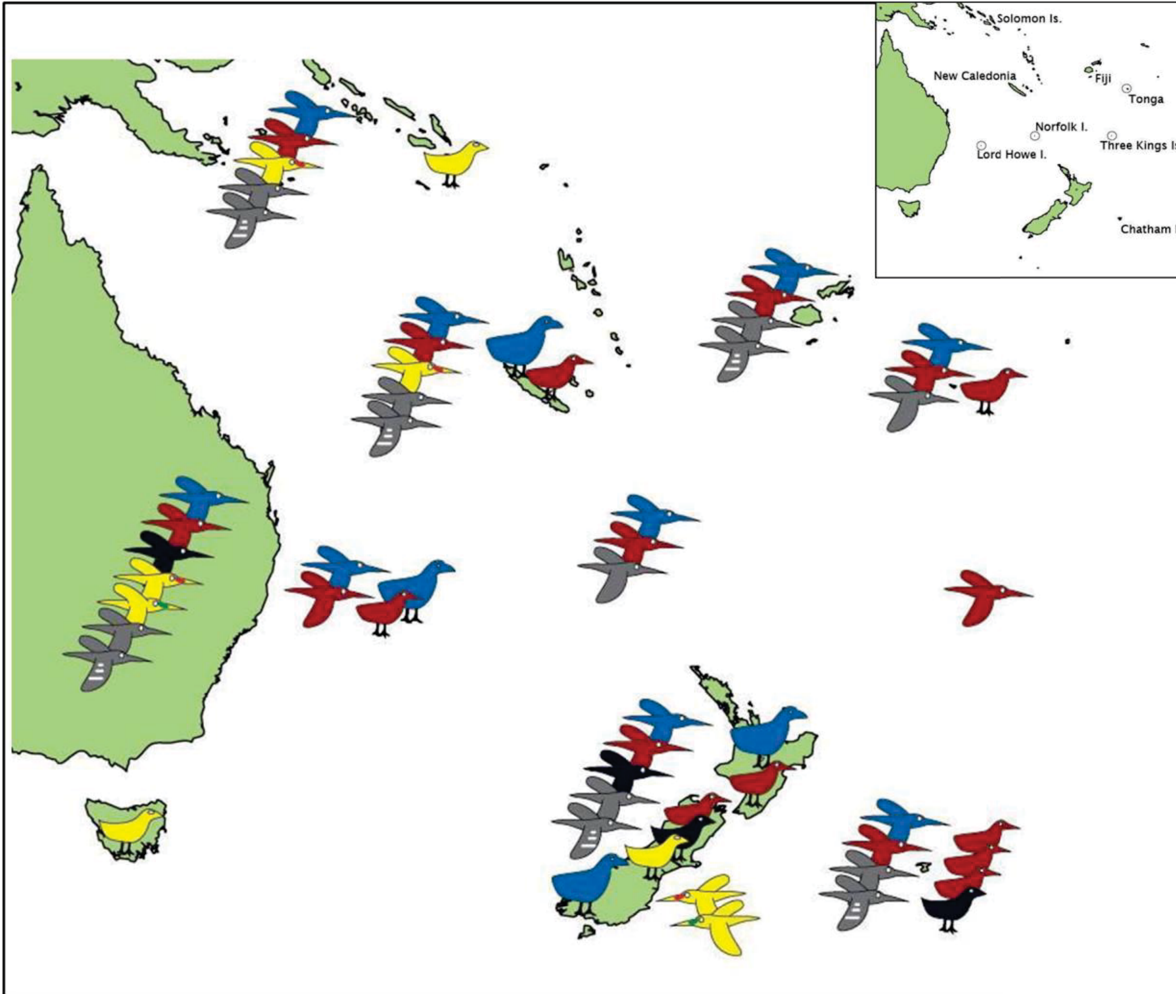


Figure 5. Multiple colonisations by rails in South-Western Pacific. Only genera found in New Zealand are represented here; flying species by winged-bird icon and flightless by walking-bird icon. Right-facing flying-birds indicate breeding populations; left-facing ones indicate vagrants. Each flightless-bird icon indicates a different endemic species in the location indicated, and each different flying-bird colour/pattern represents a different species of; *Porphyrio* blue, *Gallirallus* red, *Fulica* black, *Porzana* grey, *Gallinula* yellow. Several of the flightless species became extinct after human colonisation.

In chapter 2 "Deep global evolutionary radiation in birds: diversification and trait evolution in the cosmopolitan bird family Rallidae", the most complete species-level (~70%) time calibrated hypothesis of evolutionary relationships produced to date for Rallidae, is used to examine the mechanisms driving the diversity of this group. Sufficient breadth of taxon sampling is important to identify more realistic biological diversification processes that can reveal the degree of historical biogeographic signal and net diversification retained in the

current lineage distribution. This bird family exhibits a pattern of diversification involving episodes of range expansion and regional speciation resulting in most clades represented on all habitable continents. The results suggest that several features may have contributed to diversification rates in Rallidae. Lineage accumulation is nearly constant and morphology (frontal shield and body size), flightlessness, habitat (forest) and distribution (insular) traits are possibly associated with increasing diversification rates along with spatial and ecological processes during the Miocene and Pliocene. Diversification and the global retention of diversity have occurred in multiple lineages in Rallidae due to their dispersal ability and exploitation of ecological opportunities.

In chapter 3 "Dispersal and speciation in purple swamphens (Rallidae: *Porphyrio*)", phylogenetics and population genetics were employed to reconstruct the mode, pattern and tempo of diversification within the wide-ranging purple swamphens (genus *Porphyrio*) with emphasis in *Porphyrio porphyrio*. In this chapter I examined how dispersal along with natural selection and isolation influenced speciation rates within this lineage. I found that the clade *Porphyrio* arose during the Mid-Miocene, apparently in Africa, with a single Long-Distance Dispersal (LDD) event occurring into the Americas and several other LDD events to the North-East around 10 Mya. *Porphyrio porphyrio* was not monophyletic and several of the taxon subclades might be regarded as species. The subspecies *P. p. melanotus* appeared in Australasia during the Pleistocene (600 kya) but reached some isolated islands only in the last hundred years. Widespread occupation of oceanic islands implies high dispersal and colonization rates but gene flow probably occurs episodically and following varying routes at different times. This pattern of dispersal produces enough population differentiation that divergent natural selection can lead to potential ecological speciation.

In chapter 4 "The role of the gene flow in speciation from a colonizing rail bird in Oceania" the role of gene flow in genetic variation of the archetypal 'great speciator' banded rail (*Gallirallus philippensis*) in Oceania was examined to understand the associated factors that promote speciation within this bird lineage. Colonisation often involves marked founder effects associated with population bottlenecks that reduce within-population genetic diversity and increase genetic differentiation among populations. Studies of

fragmented areas, such as islands in archipelagos, provide important insights into the microevolutionary processes that drive the early stages of diversification. I found that the banded rail presented high genetic variability in its ancestral area or oldest native range, the Philippines archipelago, and the geographically close archipelagos Palau and Wallacea. The geographically distant and large island/continent of Australia, while somewhat lower still contained considerable genetic variability. Other archipelagos sampled were found to have less genetic diversity and closely related to haplotypes from Wallacea (Bismarck, Samoa, Vanuatu, New Caledonia, New Zealand, Cocos) and Australia (New Zealand, Samoa, Fiji, Cocos). Nucleotide diversity declined with respect to the colonisation of new archipelagos but not the haplotype diversity which remained roughly the same. However, both nucleotide and haplotype diversities are positively correlated with archipelago size area. These results are mostly concordant with abrupt genetic changes of founder events, with the Philippines as the most divergent region and Wallacea the first colonized archipelago. Subsequent and perhaps multiple colonisation events from Wallacea and Bismarck could have taking place to Australia which acted as an intermediate source for other islands in the Pacific.

The last part of this thesis is the general discussion regarding the findings of the preceding chapters within the context of avian evolution. The information obtained in the previous chapters is used to analyze features of the current perspectives on evolution of birds and propose conclusions about the applicability and further research questions based on these hypotheses. Here, the integration of ecological and historical approaches through an evolutionary framework is advocated because this big picture approach is comprehensive and necessary to explain fundamental questions of the history, diversity and distribution of life.

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Eocene diversification of crown group rails (Aves: Gruiformes: Rallidae)[1]

[1] In press: Garcia-R JC, Gibb GC, Trewick SA. PLoS ONE.

Abstract

Central to our understanding of the timing of bird evolution is debate about an apparent conflict between fossil and molecular data. A deep age for higher level taxa within Neoaves is evident from molecular analyses but much remains to be learned about the age of diversification in modern bird families and their evolutionary ecology. In order to better understand the timing and pattern of diversification within the family Rallidae I used a relaxed molecular clock, fossil calibrations, and complete mitochondrial genomes from a range of rallid species analysed in a Bayesian framework. The estimated time of origin of Rallidae is Eocene, about 40.5 Mya, with evidence of intrafamiliar diversification from the Late Eocene to the Miocene. This timing is older than previously suggested for crown group Rallidae, but fossil calibrations, extent of taxon sampling and substantial sequence data give it credence. I note that fossils of Eocene age tentatively assigned to Rallidae are consistent with my findings. Compared to available studies of other bird lineages, the rail clade is old and supports an inference of deep ancestry of ground-dwelling habits among Neoaves.

Keywords: Aves; biogeography; crown and stem ages; dating; ecological diversification; evolution.

Introduction

Hypotheses favouring Cenozoic diversification of modern bird orders after the Cretaceous–Palaeogene (K–Pg) mass extinction, inferred from the scarcity of Cretaceous fossils (Olson 1989; Feduccia 1995, 2003), have been rejected by analyses indicating the origin of several lineages during the Cretaceous (Hedges et al. 1996; Cooper and Penny 1997; Cracraft 2001; Pacheco et al. 2011; Smith et al. 2011; Jetz et al. 2012). This conclusion derives from calibration of molecular clocks (Harrison et al. 2004; Brown et al. 2008), using ever increasing genetic data, and is supported by a rising number of Cretaceous fossils (Clarke et al. 2005; Agnolin et al. 2006; Agnolin and Novas 2012). Although there is some uncertainty about phylogenetic placement, most adequate, confident and diagnostic neognath fossil material found from the early Paleogene suggests an extensive diversification of neognaths during the late Cretaceous (Mayr 2009, 2014; Mayr and Scofield 2014). Analyses using mitochondrial and nuclear data largely agree in terms of the age of the lineages leading to the main orders of modern birds (Paton et al. 2002; Paton et al. 2003; Pereira and Baker 2006; Haddrath and Baker 2012; but see Ksepka et al. 2014). Studies are nevertheless sensitive to calibration and dating tools (Ho et al. 2005), and a persistent difficulty is the influence of missing lineages on inferences regarding the timing of modern diversity (Barraclough and Nee 2001; Trewick and Gibb 2010).

In particular, studies that use data from single species to represent clades suffer from uncertain node age and placement (Hendy and Penny 1989; Holland et al. 2003), and are not informative about crown group ages. For example, recent analysis of genetic data for Palaeognathae have shed light on the ancestry of moa (Dinornithiformes) by supporting the flying South American tinamous as sister to moa within the Struthioniformes (Phillips et al. 2010; Baker et al. 2014). This finding supports a hypothesis of flying ancestors for modern day flightless Struthioniformes (Houde and Olson 1981; Houde 1986) contrary to the assumed flightless ancestry of the entire group. Separate studies support a recent radiation of moa (Haddrath and Baker 2001; Bunce et al. 2009) with their origin occurring only 5 million years ago (Pliocene). This estimated time leaves considerable disparity in the assumption that the common ancestor of species of moa was itself flightless (Tennyson et al. 2010). Similarly, raptors

or birds of prey are not monophyletic although they share a primary reliance on carnivory, either by scavenging or by capture of prey, and a number of associated functional niches (Gibb et al. 2007; Livezey and Zusi 2007; Hackett et al. 2008; Trewick and Gibb 2010). These convergent lifestyle specializations of falcons, hawks and eagles indicate a possible early group of raptors (late–Cretaceous) from which a variety of other carnivore groups have adapted to more aquatic lifestyles (Gibb et al. 2007; Livezey and Zusi 2007).

This stem versus crown age problem is common to many phylogenetic studies seeking to identify the timing of diversification within Neoaves, whether associated with location, ecology or taxonomy. Mitochondrial genes and genomes have proved valuable in avian evolutionary studies providing the means to address questions about the placement and recognition of clades within the avian phylogeny and their likely time of diversification (Pereira and Baker 2006; Slack et al. 2006; Gibb et al. 2007; Brown et al. 2008; Morgan-Richards et al. 2008; Pratt et al. 2009; Pacheco et al. 2011). These studies have mainly focused at the order level, whilst studies at family level are scarce. Recent analysis of mitochondrial DNA (mtDNA) genomes suggests that at least 30 major orders of Neoaves originated in the Cretaceous and survive to the present, but no representatives of families within Gruiformes were included (Pacheco et al. 2011).

The rail family (Aves: Rallidae) is globally distributed and extant species occupy niches associated with terrestrial and freshwater habitats. Among these predominantly ground dwelling and foraging birds are a high frequency of flightless species (Olson 1973; Livezey 2003). The ecology and geographic origins of modern rails are almost entirely unknown but most of the putatively “primitive” species, as well as several distinctive genera, inhabit forests of the Old World tropics (Olson 1973). Fewer genera are found in the New World, and most of these have been interpreted as being derived from an Old World stem (Olson 1973). Some genera (e.g. *Rallus* and *Fulica*) appear to have specialized and radiated in the Americas before reinvading the Old World (Olson 1973). There are 135–143 currently recognized species within 33–40 genera (Taylor 1998; Clements et al. 2012; Lepage 2013), only four of which (*Porzana, Porphyrio, Gallinula*, and *Fulica*) have a worldwide distribution (Figure 1). *Porzana*, as currently recognized,

is the most speciose genus in the family (11.9% of rails are currently classified as *Porzana*), followed by *Gallirallus* (11.2%), *Fulica* (7.7%), and *Gallinula, Laterallus, Rallus, Amaurornis* and *Sarothrura* (6.3% each). Molecular analysis shows that *Sarothrura* belongs to a separate lineage from Rallidae, Sarothruridae, and more closely related to the family Heliornithidae (Sibley and Ahlquist 1990; Slikas et al. 2002; Fain et al. 2007; Hackett et al. 2008), but current taxonomy and bird lists still retain this lineage within Rallidae (Taylor 1998; Clements et al. 2012). Most of the diversity of *Porzana* and *Gallirallus* is found in Asia and Oceania (Figure 1). Asia also contains three endemic genera (*Aramidopsis, Habroptila* and *Gallicrex*) as does Oceania (*Nesoclopeus, Eulabeornis* and *Megacrex*). All of those genera except *Nesoclopeus* are monotypic. Africa has 16 endemic species in seven endemic genera (including *Atlantisia* in the South Atlantic islands), 54 species occur in America, including 27 in eight endemic genera and six of the nine *Rallus* species, while only nine species occur naturally in Europe.

Spatial, morphological and current phylogenetic information suggests that rails may be are old within the Neoaves. Analyses indicate a deep placement of the lineage but the inference is based on limited species representatives and short DNA sequences, which requires better resolution (Ericson et al. 2006; Brown et al. 2007; Fain et al. 2007; Hackett et al. 2008; Houde 2009; Jetz et al. 2012). In the present study, I use complete mitochondrial genomes to assess temporal diversification within Rallidae. I further compare my estimates with those available for other extant bird lineages in order to shed light on biogeographic/spatial patterns operating in diversification within Neoaves.

Materials and Methods

Sampling

The data set compiled new assembled mitochondrial genomes of six species within Rallidae and one species within Heliornithidae plus five published rail mitochondrial genomes. To maximize lineage diversity I selected species using available geographic ecological and phylogenetic information. I include three widespread and flying representatives associated with wetland and grassland areas: *Fulica atra* (common coot; KF644582), *Gallinula chloropus* (common moorhen; HQ896036), and *Porphyrio*

porphyrio (purple swamphen; KF701062). *Coturnicops exquisitus* (Swinhoe's rail; NC012143) found in wetlands and *Rallina eurizonoides* (slaty–legged crake; NC012142) inhabiting forests are both volant species present in Asia. *Gallirallus philippensis* (banded rail; KF701061) is distributed in Asia and Oceania, and *Eulabeornis castaneoventris* (chestnut rail; KF644583) in Oceania and both are flying species that occupy wetlands. *Gallirallus okinawae* (Okinawa rail; NC012140), is found endemic to wet forest on Okinawa island in the Japanese archipelago. *Gallirallus australis* (weka; KF701060) and *Porphyrio hochstetteri* (takahe; EF532934) endemic to New Zealand, and *Lewinia muelleri* (Auckland rail; KF644584) is endemic to the subantarctic Auckland Islands. *Gallirallus australis, P. hochstetteri* and *L. muelleri* live in mixed forest and grassland habitats. *Gallirallus okinawae, G. australis* and *P. hochstetteri* are absolutely flightless while *L. muelleri* is reported to fly well but infrequently.

mtDNA genomes

Sample tissue details can be found in supplementary Table S1. Taking into account the already available mtDNA genomes of rails in GenBank I chose these species because of their geographical range in the Southern Hemisphere (e.g. *E. castaneoventris* and *L. muelleri*) and the relationships among genera. It has been inferred from molecular phylogenetics that Grues (suborder comprising Rallidae, Gruidae, Heliornithidae, Aramidae, Psophiidae and Aptornithidae) has a palaeo-austral signature (Fain et al. 2007; Houde 2009) but the fossil record is mainly found in the Northern Hemisphere. Although the intrafamiliar relationships in Rallidae are mostly unknown, I sought to include representatives of different and more distant genera in the family following Olson (1973). *Heliornis fulica* (sungrebe) was included as a close outgroup (Hackett et al. 2008; De Pietri and Mayr 2014). I used a modified phenol–chloroform procedure (Sambrook et al. 1989) involving digestion in CTAB buffer for genomic DNA extraction. Genome DNA extractions were verified by gel electrophoresis and quantified using Qubit 2.0. An estimated 2–10 ng of each DNA was subjected to Whole Genome Amplification (WGA) via next generation sequencing (NGS) using the Illumina HiSeq platform (Beijing Genomics Institute, BGI) with 100 bp paired–end

reads. Library preparation for sequencing was as described by Shendure and Ji (2008) and Mardis (2008).

Sequences quality, mapping and assembly

For quality control of the fastq files I used FastQC v0.10.1 (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc) which helps to identify clusters with a low signal and low–quality base calls based on score value chastity ≥ 0.6. Contigs were created using *de novo* assembler Velvet v1.1.06 (Zerbino and Birney 2008) which has been developed for assembly of short read using a Brujin graph algorithm. I conducted assemblies of the paired reads using multiple hash lengths (k = 43, 53, 63, 73, 83) and assembled the contigs obtained from the best kmer lengths (generally around 73). All the assemblies were performed on a server with 72 cores and 144 Gb access memory. Sequences were mapped using Geneious v6.0.5 (Drummond et al. 2012a) with reference to the previously published mtDNA genomes of Okinawa rail, GenBank accession number NC012140 (Ozaki et al. 2010) and common moorhen, GenBank accession number NC015236 (Kan and Li), and visualized in Tablet v1.11.08.10 (Milne et al. 2010). New mtDNA genomes were submitted to GenBank (Table S1).

Phylogenetic analyses

For phylogenetic analyses, mtDNA genomes of additional Neognathae species were downloaded from GenBank. These lineages were from closely related groups to Rallidae (e.g. Gruidae, Otididae, Cuculidae) and provide appropriate context for dating analyses (Table S1). Galloanserae species were used as a known outgroup to all these taxa (Hackett et al. 2008). Several studies (Hackett et al. 2008; Morgan-Richards et al. 2008; Yang et al. 2010) have shown that the kagu (*Rhynochetos jubatus*) is not, despite some morphological and behavioural similarities, grouped within the Gruiformes; therefore this species was not included in the analyses.

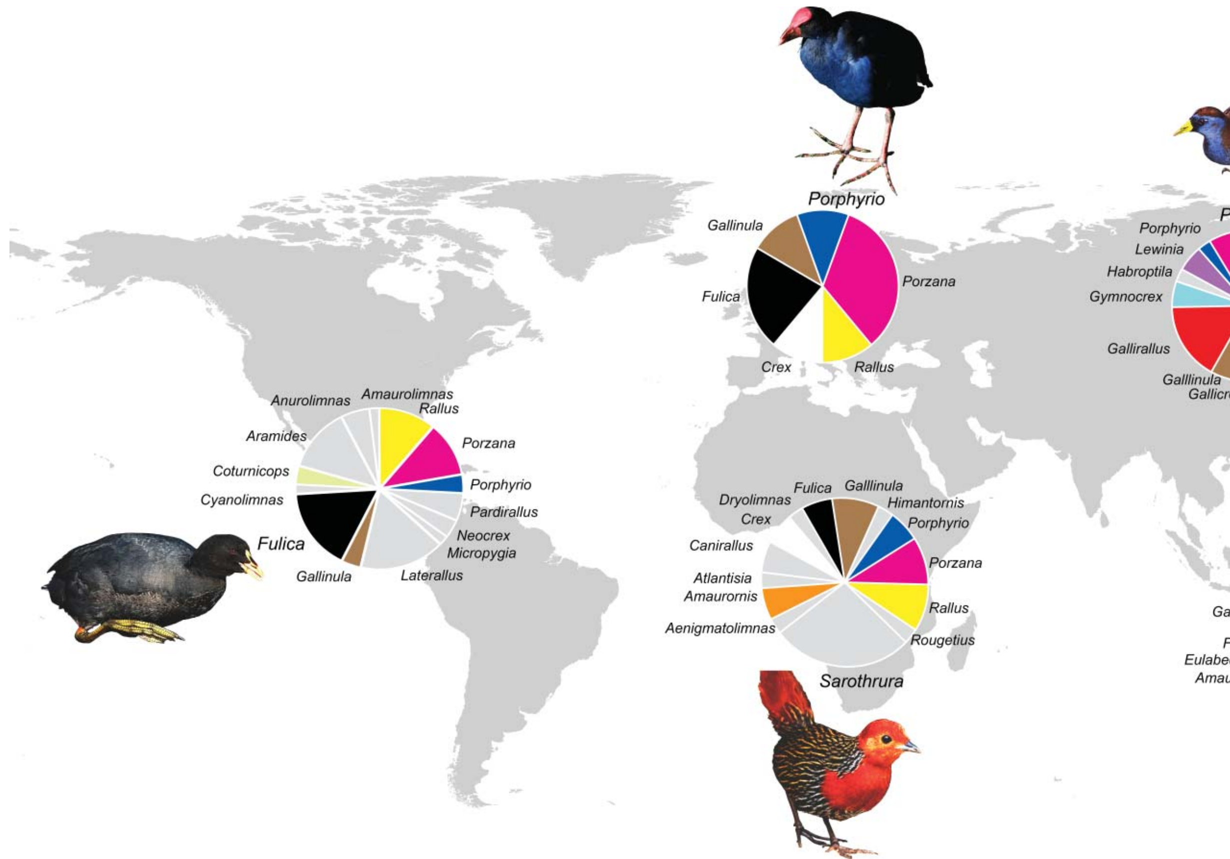

Figure 1. Proportional diversity of genera in Rallidae by geographic regions (Continents). Genera endemic to each continent are region are indicated by identical colour in each pie chart. Taxonomic treatment follows Taylor (1998) and Clements (2012), *Sarothrura* outside the family (see Fain et al. 2007 and Hackett et al. 2008).

Alignment of the mitochondrial sequences was performed with Geneious v6.0.5 (Drummond et al. 2012a) using manual adjustment. Each gene alignment was checked prior to phylogenetic analysis. I partitioned the aligned genomes into protein-coding genes, tRNAs, rRNAs, and noncoding fragments (including the origin of replication and the hypervariable region) (Harrison et al. 2004). I further partitioned the protein-coding genes based on amino acid sequences, into stems and loops data for rRNAs (Gutell et al. 1993; Springer and Douzery 1996) and cloverleaf pattern for tRNAs, which correspond to RNA secondary structures of those genes. Protein-coding genes were aligned manually based on the deduced amino acid sequences. The alignments of rRNA and tRNA genes were corrected by excluding ambiguous positions, such as loops and indels. Stop codons and ambiguous alignments next to gaps (conserved amino acid and RNA stems defined the inclusion boundaries of ambiguous regions next to gaps) were excluded from the alignment. The Control Region and NADH6 were excluded from the analyses due to alignment instability and heterogeneous base composition which can confound phylogenetic inferences.

The total length of the analysed mitogenomic dataset was 13,768 nucleotides which included the following partition scheme (Harrison et al. 2004): 1) first–codon position of the 12 protein–coding genes and 2) second–codon position of the 12 protein–coding genes, 3) RY–coding at the third–codon position of the 12 protein–coding genes, 4) loops of the tRNAs and rRNA combined, 5) stems of the tRNAs and rRNA combined. I performed all subsequent analyses with this partition strategy. Phylogenies were inferred using Bayesian Markov Chain Monte Carlo (MCMC) as implemented in MrBayes (Ronquist and Huelsenbeck 2003) with 20 million generations sampled every 2000 generations and a general time reversible model with gamma distribution (GTR + Γ) model of evolution. The model was estimated in ModelTest v3.7 using the Akaike Information Criterion (Posada and Crandall 1998). Convergence and diagnostics of the Markov process were evaluated by the stability of parameter estimates across generations using Tracer v.1.6 (http://tree.bio.ed.ac.uk/software/tracer/). A burn in of 10% gave optimal results. I obtained Effective Sample Sizes (ESS) above 200 for all parameters. Maximum Likelihood (ML) with rapid bootstrapping was implemented in RAxML using GTR + Γ. Analyses were performed via the Cipres portal (Miller et al.

2010) and trees were viewed in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) and SplitsTree v4.12.8 (Huson and Bryant 2006).

Time of divergences

Divergence times were estimated using a Lognormal relaxed Bayesian clock implemented in BEAST v1.7.5 (Drummond et al. 2012b). A lognormal distribution was chosen because this shape accommodates greater flexibility regarding a cladogenetic event (Ho and Phillips 2009; Brown and van Tuinen 2011). For calibration constraints I used Galloanserae (Benton and Donoghue 2007; Benton et al. 2009) with a normal distribution of 66–86 Mya (95% range) and the stem sphenisciform *Waimanu* (Slack et al. 2006) with a normal distribution of 61.5–65.5 Mya (95% range). The *Waimanu* stem Penguin is without doubt the oldest specimen within the Sphenisciformes currently known (Fordyce and Jones 1986; Fordyce and Jones 1990; Slack et al. 2006) and *Vegavis*, the oldest reliable Galloanserae (anseriform) that is preserved in sufficient quality to provide contrasting anatomical characteristics with living birds (Clarke et al. 2005; Benton et al. 2009; O'Connor et al. 2011). These well–documented fossils provide confidence about their phylogenetic placement and serve together as phylogenetically external calibration points for estimation of divergence dates within neognaths and Gruiformes. They are considered to be reliable time constraints and have been widely used for divergence estimations because of the high confidence about their placement (Ho and Phillips 2009). While there is some disagreement on the precise placement of *Vegavis* (Elzanowski and Stidham 2011) within anseriforms, it demonstrates the existence of galloanserine birds in the late Cretaceous (Elzanowski and Stidham 2011) and of extensive diversification and phylogenetically separated neognath lineages in the late Cretaceous/early Paleogene time (Mayr 2009, 2011; Mayr and Scofield 2014).

I combined the results of three independent runs with 30 million generations and a burn–in of 10% for each. Chains were sampled every 5000th generation and a Birth–Death process prior was used for the speciation model (Rannala and Yang 1996). The analysis used a starting tree topology from the ML analysis modified with approximate years for branch lengths using TreeAnnotator (Drummond et al. 2012b). I obtained for

each run ESS above 200 for 98% of the parameters. The tree with the times of divergence and highest posterior density (HPD) intervals was visualized using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Phylogenetic analyses

Inferred phylogenies and nodal support based on Bayesian and ML analyses were concordant and the tree topology (Figure 2) was congruent with trees previously inferred using nuclear and mitochondrial sequences (Fain et al. 2007; Hackett et al. 2008; Yang et al. 2010). In my analysis Rallidae was sister to Heliornithidae, and this clade in turn was sister to Gruidae (Fain et al. 2007). I obtained similar levels of statistical support from ML and Bayesian analyses for internal groups within Rallidae with the expected pattern of intra–generic clusters (*Gallirallus* and *Porphyrio*). I found that *Eulabeornis castaneoventris* was placed within the genus *Gallirallus* and *G. australis* arises from the base of this clade (Kirchman 2009; Kirchman 2012). *Fulica atra* and *Gallinula chloropus* form a sister group, and *Coturnicops exquisitus* and *Rallina eurizonoides* form a sister clade to *Porphyrio*.

Divergence times

The long branch leading to *Heliornis fulica* (see Figure 2), might result from heterogeneity of mutation rates among lineages that can influence phylogenetic topology and time estimations. To allow for this possibility I estimated the times of divergence both with and without *H. fulica*, with similar results returned by Bayesian MCMC analyses. I report here the results including *H. fulica* (Figures 3 and S1). The split of Grues into rail–like and crane–like lineages is supported as occurring around the boundary of K–Pg mass extinction 66 (74–59) Mya (Houde 2009). The estimated time of divergence of the Heliornithidae and Rallidae (Ralloidea) lineages was 52 (60–44) Mya. My divergence time analyses for crown group Rallidae is post K–Pg occurring during the Eocene about 40.5 (49–33) Mya. Among representatives of Rallidae included in this analysis there was evidence of several relatively early lineage-splitting events during the Late Eocene and Early Oligocene. Estimations of the divergence times obtained in this study indicate a common ancestor for *Gallirallus* and *Lewinia* around

28 (36–21) Mya, a divergence time of *Rallina* and *Coturnicops* around 29 (37–21) Mya, and origination of the aquatic *Fulica* and *Gallinula* clade 18 (26–10) Mya.

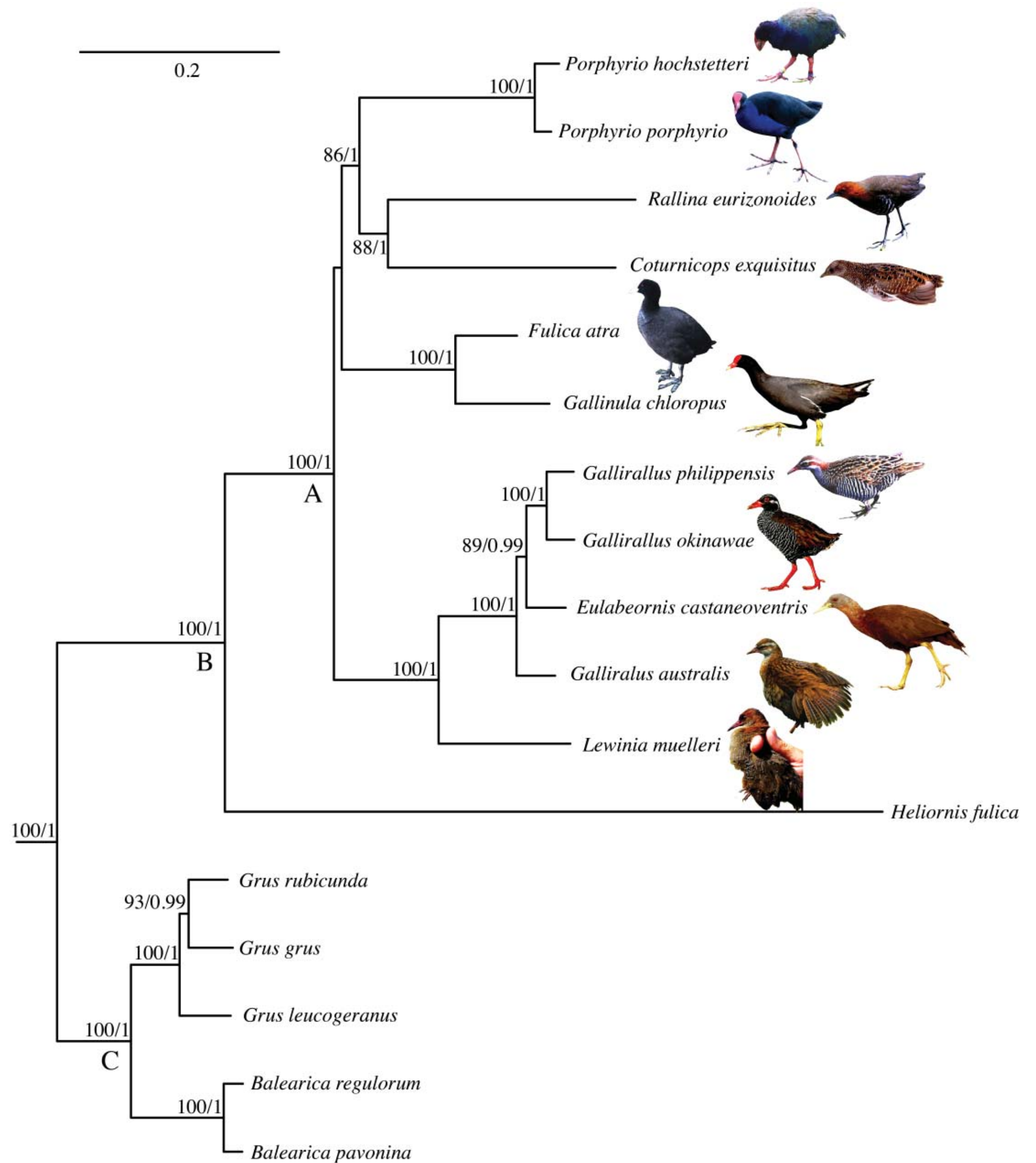

Figure 2. Maximum Likelihood tree resulting from analysis of complete mitochondrial genomes of birds with an emphasis in Rallidae. Species outside of the Grues are not shown. Bootstrap support over 70% and Bayesian posterior probabilities over 0.9 are indicated in each branch. Letters below the nodes refer to the families within the Order Gruiformes included in this study (A = Rallidae, B = Heliornithidae, C = Gruidae).

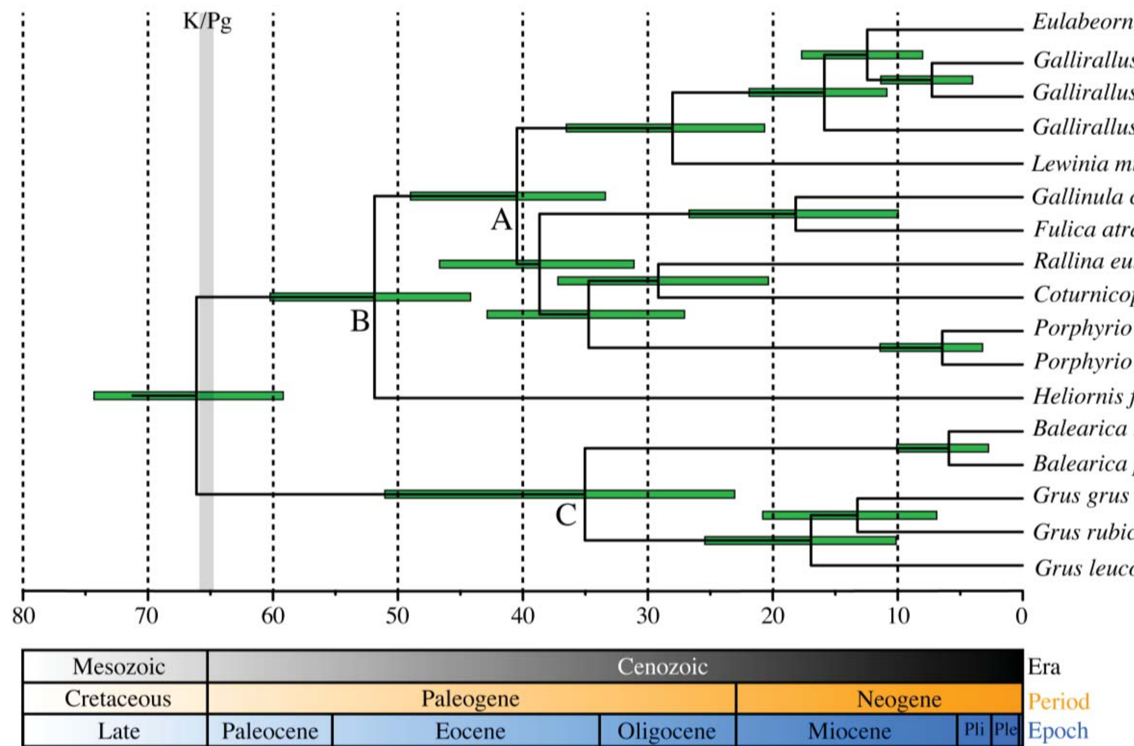

Figure 3. Chronogram based on analysis of complete mitochondrial genomes with a Lognormal relaxed–clock Bayesian ana established by calibration fossils of Galloanserae with a minimum age of 66 Mya and maximum age of 86.5 Mya and Sphenisci 65.5 Mya. For each node the estimate time of divergence is indicate and the green bar represents the 95% HPD intervals of node ago (Mya) and geological eras, periods and epochs are indicate where Pli is Pliocene and Ple is Pleistocene. A complete figure in found in supplementary Figure S1. Bootstrap support, Bayesian posterior probabilities and letters referring families within order G

Discussion

Molecular phylogeny

My phylogenetic analysis of bird mitogenomes is consistent with inferences made from several studies using fewer loci and provides strong corroboration of phylogenetic hypotheses and observed pattern of the molecular rate of evolution (Fain et al. 2007; Hackett et al. 2008). The topology is consistent with that of Fain et al. (Fain et al. 2007), and relative branch lengths are similar with respect to the short internal branches in Gruidae and the comparatively long branches in Rallidae/Heliornithidae (Fain et al. 2007; Hackett et al. 2008). The disparity in length of these branches is detected in both nuclear and mitochondrial data, suggesting it reflects real variation in the rates of molecular evolution among clades (Fain et al. 2007). Rails contribute approximately 85% of the species in Grues diversity and this high level could potentially be attributable to a relatively fast mutation rate (Eo and DeWoody 2010; Lanfear et al. 2010). The scarcity of heliornithids (three species) contradicts this idea but the disparity in species numbers could be due to nonuniform diversification rates through time and/or uneven extinction rates (Purvis et al. 2000; Alfaro et al. 2009).

Origin and evolution of rails

Previous studies have addressed the question of the origin of the Gruiformes (Fain et al. 2007; Houde 2009) and the biogeography and evolution of some lineages of rails have been recently considered (Trewick 1997; Slikas et al. 2002; Kirchman 2012; Ruan et al. 2012). Nevertheless, identification of the basal split and diversification of the rails has remained uncertain because of differences in the estimated dates and approaches used (Ericson et al. 2006; Brown et al. 2007; Fain et al. 2007; Houde 2009).

I find the width of the 95% HPD intervals for Heliornithidae–Rallidae clade divergence and the crown–group Rallidae in my analysis overlap with interval age estimations reported from nuclear genes by Fain (2007), Houde (2009), and Brown (2007). However, the mean estimates of Heliornithidae–Rallidae divergence and basal Rallidae in the present analysis were much older than mean estimations in those studies (about twice the age). The observed tendency of analyses using mtDNA to overestimate node age compared to nuclear markers might be the source of this discrepancy (Ksepka et al.

2014), and it has been inferred that mtDNA data tends to result in overestimated ages of shallower nodes in particular (Brown and van Tuinen 2011; Ksepka et al. 2014). To minimise any potential overestimation of “shallower” nodes (compared to the nodes used to calibrate the tree) I applied several recommended strategies: 1) a partition scheme including RY–coding at the third–codon position; 2) relaxation of the molecular clock without assuming rate correlation among branches and; 3) variation across sites with GTR model and gamma distribution. I find that my estimations of “deeper” node ages (e.g. Ralloidea–Gruoidea divergence) are in fact very similar to those reported using nuclear data (Houde 2009). Although it appears that “shallower” node ages might be overestimates in my analysis (node A of the Rallidae lineage and node B of the Heliornithidae–Rallidae divergence in Figure 3), I have similar estimates for Gruinae–Balearicinae (node C in Figure 3) to other studies using nuclear (Fain et al. 2007) and mitochondrial data (Krajewski et al. 2010) and different calibration constraints. This suggests there is no systemic tendency for overestimation of late Eocene nodes. More probably, disparity with previous molecular analysis relates to difference in calibration constraints and taxon sampling of my study and others. For instance, Fain et al. (Fain et al. 2007) used as constraints external to Grues a lower (30 Mya) and upper (45 Mya) bound of the alcid-larid split.

Fossils of cranes (Gruidae) have been reported from the middle Eocene in Europe (Olson 1985; Mayr 2005; Lindow and Dyke 2006), and the earliest sungrebe fossil record is from the middle Miocene (14 Mya) in North America (Olson 2003). Two Paleogene Ralloidea fossils designated as *Messelornis* and *Walkbeckornis* are consistent with my estimated age of Ralloidea around 52 (60–44) Mya (Mayr 2009, 2014). The estimated time in my study for the common ancestor of living rallids is about 7 million years older than existing fossils assigned to the crown group. However, my lower interval value is consistent with European fossils within *Belgirallus* from the late Eocene–early Oligocene that have been suggested as representing the earliest Rallidae (Olson 1977; Mayr and Smith 2001; Mayr 2006). Recent examination of the humerus, coracoid and tarsometatarsus led to the proposal that *Belgirallus* belongs to stem group Ralloidea closely related to *Palaeoaramides* from the late Oligocene–early Miocene (De Pietri and Mayr 2014). Nevertheless, great caution is needed in attribution of

stem/crown group fossils when the availability of suitable comparisons is limited, systematics of the group is uncertain, and morphological characters can mislead phylogeny (Dyke and Van Tuinen 2004; Fountaine et al. 2005; Livezey and Zusi 2007; Dyke and Crowe 2008). The current absence of suitable fossils from the Eocene does not demonstrate that a common ancestor of living Rallidae did not exist at that time, and indeed some have been tentatively attributed to the family. *Palaeorallus, Eocrex* or *Fulicaletornis* from the Early Eocene in North America (Wetmore 1931; Olson 1977) or rail–like taxa of the genus *Songzia* from the Early Eocene in China (Hou 1990; Wang et al. 2012) might represent extinct crown group rails, but their placement must remain equivocal (Mayr 2005, 2009) because of the fragmentary nature of the specimens. The fossils at least hint that extinct species that share common ancestry with the living lineages in my analysis might have existed. As with the continuing discovery of Cretaceous bird fossils (Stidham 1998; Clarke et al. 2005; Fountaine et al. 2005; Brocklehurst et al. 2012), it is likely that better rail and Grues specimens will be forthcoming.

Crown age of bird lineages

Studies of the origin and diversification of crown bird lineages provide insights into the rates and modes of ecological speciation. Comparisons of data from studies of birds makes it very clear that stem and crown group ages are not correlated, which is expected where speciation and extinction rates are uneven over time. For instance, several studies using complete mtDNA genomes or gene sequences show a relatively recent diversification of passerine and non–passerine bird lineages (Bunce et al. 2009; Jønsson et al. 2010; Krajewski et al. 2010; Lerner et al. 2011; White et al. 2011), with most crown lineages appearing during the Neogene (Figure 4), while taxa based on fossils assigned as part of stem groups are much older or younger than molecular date estimations (Mayr 2005; Mayr and Manegold 2006; Worthy et al. 2007; Mayr 2009; Tennyson et al. 2010; Mayr 2013). However, assessment of radiations in birds must be characterized by their geographical settings (Jetz et al. 2012) because the spatial context of family level diversification is highly variable. For example, extant honeycreepers (family Fringillidae) in Hawaii (Lerner et al. 2011) and whistlers (family Pachycephalidae) in the Indo–Pacific (Jønsson et al. 2010) represent recent insular

radiations apparently responding to local ecological opportunities and climatic variations. Within the Palaeognaths, the extinct New Zealand moa radiation is classified in three families (Bunce et al. 2009). Available evidence indicates Pliocene diversification within Dinornithiformes; even if treated as a single New Zealand family, the moa clade shows a shallow insular radiation (Figure 4).

Insular lineages appear to have relatively shallow crown ages even though some archipelagos are comparatively old (Johnson et al. 1989; Baldwin and Sanderson 1998; Fleischer et al. 1998), whereas lineages that achieved wider distributions have deeper ages. For instance, widespread parrots (family Cacatuidae) (White et al. 2011), cranes (family Gruidae) (Krajewski et al. 2010) and rails (family Rallidae) have substantially deeper history (Figure 4). This indicates that a larger spatial range might increase the probability of lineage survival. The remarkable capacity of the rails to colonise and adapt to a wide variety of habitats perhaps favoured the retention of lineages through time. Rails show a fantastic capacity and propensity for range expansion and local adaptation with instances of supertramp species, such as *P. porphyrio* and *G. philippensis* (Diamond 1974; Mayr and Diamond 2001), which have colonized remote archipelagos in the Pacific (Trewick and Gibb 2010). However, the group has mainly retained a sedentary–ground walking ecology. Many lineages within Rallidae are not specialised to narrow marginal habitats but have proved resilient throughout the globe in diverse conditions. It seems likely that the temporal resilience of Rallidae and other cosmopolitan bird lineages has been guided by spatial and ecological plasticity. Further analysis with additional sampling will help reveal to what degree historical biogeographic signal has been retained in the current lineage distribution.

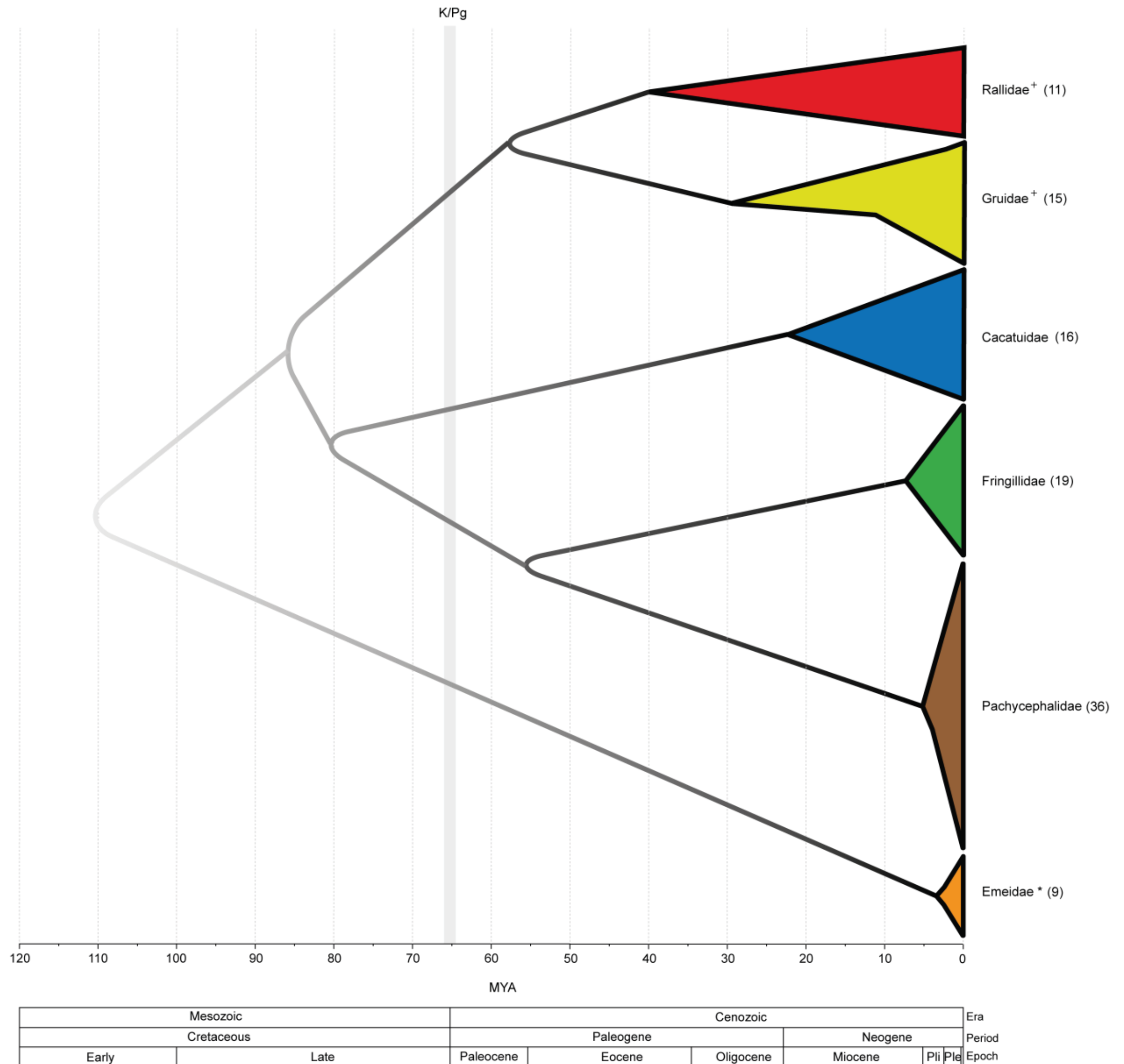

Figure 4. A schematic diagram representing available temporal patterns of diversification in bird families: Rallidae (this study), Gruidae (Krajewski et al. 2010), Cacatuidae (White et al. 2011), Fringillidae (Lerner et al. 2011), Pachycephalidae (Jønsson et al. 2010) and Emeidae (Bunce et al. 2009). The number of species included in each study (in parenthesis) is represented by the respective clade height at zero time. Time of diversification among clades follows the most complete molecular time estimations on bird fauna (Jetz et al. 2012). Intensity of branch colour reflects the degree of confidence from available analyses that use various dating approaches. Cross symbol indicates studies using complete mitochondrial genomes to estimate divergence times. Asterisk indicates inclusion of Megalapterygidae and Dinornithidae species within Emeidae.

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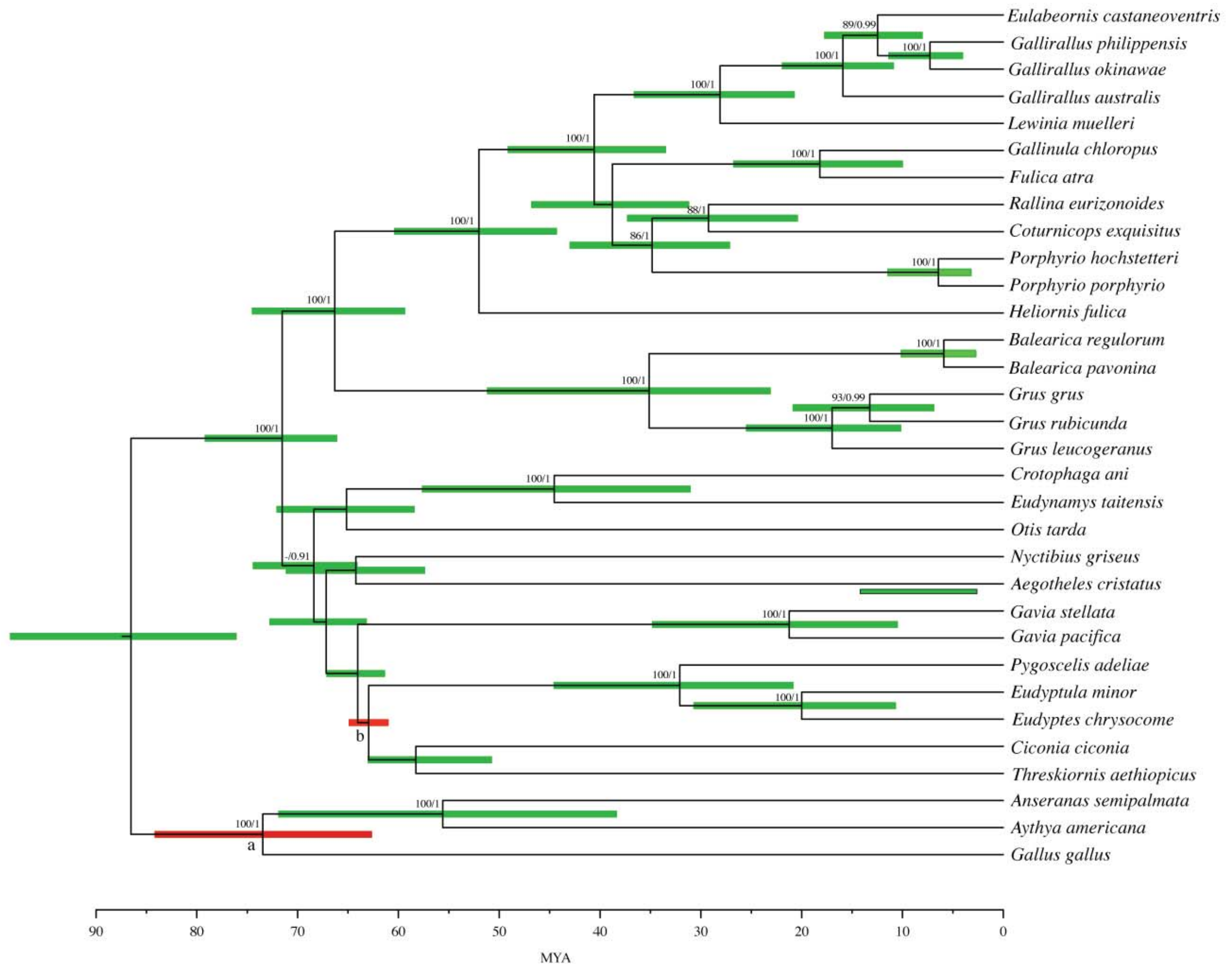
Table S1. Taxa, Family and Order, museum voucher numbers, type of tissue, specimen sampling locality, GenBank ac
data of the mtDNA genomes included in this study. N/A = Not Available. Acronyms for museums are: ANWC = A
Australia; MZUSP = Museu de Zoologia da Universidade de São Paulo (Brazil)

Species	Family/Order	Museum voucher	Type of tissue	Sample locality	Ge
Coturnicops exquisitus	Rallidae/Gruiformes	N/A	N/A	N/A	N
Eulabeornis castaneoventris	Rallidae/Gruiformes	ANWC50493	Muscle	Australia	K
Fulica atra	Rallidae/Gruiformes	ANWC50980	Muscle	Australia	K
Gallinula chloropus	Rallidae/Gruiformes	N/A	N/A	N/A	H
Gallirallus australis	Rallidae/Gruiformes	N/A	Muscle	New Zealand	K
Gallirallus okinawae	Rallidae/Gruiformes	N/A	N/A	N/A	N
Gallirallus philippensis	Rallidae/Gruiformes	ANWC32326	Muscle	Australia	K
Lewinia muelleri	Rallidae/Gruiformes	N/A	Blood	New Zealand	K
Porphyrio hochstetteri	Rallidae/Gruiformes	N/A	N/A	New Zealand	E
Porphyrio porphyrio	Rallidae/Gruiformes	N/A	Muscle	New Zealand	K
Rallina eurizonoides sepiaria	Rallidae/Gruiformes	N/A	N/A	N/A	N
Heliornis fulica	Heliornithidae/Gruiformes	MZUSP79862	Muscle	Brazil	K
Balearica pavonina	Gruidae/Gruiformes	N/A	N/A	N/A	F
Balearica regulorum	Gruidae/Gruiformes	N/A	N/A	N/A	F
Grus grus	Gruidae/Gruiformes	N/A	N/A	N/A	F
Grus leucogeranus	Gruidae/Gruiformes	N/A	N/A	N/A	F
Grus rubicunda	Gruidae/Gruiformes	N/A	N/A	N/A	F
Otis tarda	Otididae/Otidiformes	N/A	N/A	N/A	F
Aegotheles cristatus	Aegothelidae/Caprimulgiformes	N/A	N/A	N/A	E

Nyctibius griseus	Nyctibiidae/Caprimulgiformes	N/A	N/A	N/A	H
Crotophaga ani	Cuculidae/Cuculiformes	N/A	N/A	N/A	H
Eudynamys taitensis	Cuculidae/Cuculiformes	N/A	N/A	N/A	E
Ciconia ciconia	Ciconiidae/Ciconiiformes	N/A	N/A	N/A	A
Threskiornis aethiopicus	Threskiornithidae/Ciconiiformes	N/A	N/A	N/A	G
Eudyptes chrysocome	Spheniscidae/Sphenisciformes	N/A	N/A	N/A	A
Eudyptula minor	Spheniscidae/Sphenisciformes	N/A	N/A	N/A	A
Pygoscelis adeliae	Spheniscidae/Sphenisciformes	N/A	N/A	N/A	K
Gavia pacifica	Gaviidae/Gaviiformes	N/A	N/A	N/A	A
Gavia stellata	Gaviidae/Gaviiformes	N/A	N/A	N/A	A
Aythya americana	Anatidae/Anseriformes	N/A	N/A	N/A	A
Anseranas semipalmata	Anseranatidae/Anseriformes	N/A	N/A	N/A	A
Gallus gallus	Phasianidae/Galliformes	N/A	N/A	N/A	A

Figure S1. Chronogram showing all species analysed in this study. Divergence times are based on analysis of complete mitochondrial genomes with a relaxed–clock Bayesian analysis using BEAST. Bootstrap support over 70% and Bayesian posterior probabilities over 0.9 are indicated on each branch. Calibration constraints used to estimate divergence times are shown as red bars where a = calibration fossil of Galloanserae with a minimum age of 66 Mya and maximum age of 86.5 Mya, and b = calibration fossil of Sphenisciformes with an age range from 61.5 Mya to 65.5 Mya.



Deep global evolutionary radiation in birds: diversification and trait evolution in the cosmopolitan bird family Rallidae[1]

[1] In press: Garcia-R JC, Gibb GC, Trewick SA. Molecular Phylogenetics and Evolution.

Abstract

Sufficient breadth of taxon sampling in major organisms groups is important to identify more realistic biological diversification processes that reveal the degree of historical biogeographic signal and net diversification retained in the current lineage distribution. I examine the mechanisms driving diversity in one of the major avian clades with an exceptional large–scale radiation, the family Rallidae, using the most complete species–level (~70%) time calibrated hypothesis of evolutionary relationships produced to date. I find that Rallidae exhibit a pattern of diversification involving episodes of range expansion and regional speciation that results in most clades represented in all habitable continents. My results suggest that several features may have played an important role on the diversification rates in Rallidae. Lineage accumulation is nearly constant and morphology (frontal shield and body size), innovate (flightlessness), habitat (forest) and distribution (insular) traits are possibly associated with increasing diversification rates along with spatial and ecological processes during the Miocene and Pliocene. Diversification and the global retention of lineage diversity have occurred in multiple lineages in Rallidae due to their dispersal ability and exploitation of ecological opportunities.

Keywords: adaptation; biogeography; convergence; ecology; phylogeny; radiation; Rallidae; taxonomy.

Introduction

Unravelling the evolutionary ecology of birds requires extensive sampling of large phylogenetic groups comprising species that span several regions. Linking phylogenetics with analysis of speciation patterns and processes is hugely challenging, primarily because increasing phylogenetic time is accompanied by uncertainty about what taxa have existed and incomplete phylogenies are apt to give false or at least doubtful impressions of diversification rates (Crisp and Cook 2009). Young and often abrupt species radiations provide compelling evidence of shallow ancestral traits. Island radiations routinely reveal decreased gene flow and exposure to an array of novel environmental conditions as drivers of diversification. Spatial isolation, environmental change and/or ability to colonize new habitats are all implicated in behavioural and physical adaptations (e.g. moa, Bunce et al. 2009; whistlers, Jønsson et al. 2010; honeycreepers, Lerner et al. 2011; vangas, Jønsson et al. 2012) . Many empirical studies focus on radiations within archipelagos because of the opportunity to sample the majority of relevant extant species within well defined geographical boundaries (Moyle et al. 2009; Jønsson et al. 2012), and such systems provide fairly clear evidence about likely mechanisms influencing speciation. However, understanding of the mechanisms promoting diversification in older radiations across larger landscapes is generally less well informed (but see ovenbirds and woodcreepers, Derryberry et al. 2011; auks, Weir and Mursleen 2013).

The evolutionary history of one of the most speciose clades of birds, the cosmopolitan family Rallidae, is not well known. This diverse family comprises between 135 and 148 recognized species, constituting approximately 1.3% of extant birds and 85% of Gruiform diversity, within 33–40 genera (Taylor 1998; Houde 2009; Clements et al. 2012), of these 39% are monotypic (Supplementary material –Figure S1). Some members of this family possess a noted tendency to colonize oceanic islands and evolve insular and sometimes flightless species (Olson 1973b; Ripley 1977). At least 32 species are known to have this condition (Taylor 1998; Livezey 2003), providing a model system for studies in flightless evolution (Trewick 1997a; Kirchman 2009; Kirchman 2012).

Because rails have encountered and adapted to similar environments across their geographic range, they have been subject to convergence that has hampered the

understanding of their evolutionary origins, relationships, biogeography and diversification processes. This is reflected in the unstable taxonomy of the group. A large proportion of species have been placed in several different genera at one time or another as various morphological and ecological information is brought to bear on their systematics, whilst others have been abandoned in monotypic genera. Numerous rearrangements in the classification have been suggested in the last two centuries based on morphological characters (e.g. Sharpe 1894; Peters 1934; Berndt and Meise 1960; Fisher and Peterson 1964; Olson 1973a; Ripley 1977; Livezey 1998), while subgroups of their diversity have received some attention with molecular data (e.g. Sibley et al. 1993; Trewick 1997a; Slikas et al. 2002; Groenenberg et al. 2008; Kirchman 2009; Tavares et al. 2010; Goodman et al. 2011; Kirchman 2012; Ruan et al. 2012). However, no molecular study has had sufficient breadth of taxon sampling to resolve the core problems thoroughly. Previous studies have included no more than 20 rail species, about 14% of the diversity, only a single species per genus and/or too few relevant genera (e.g. Trewick 1997a; Slikas et al. 2002; Kirchman 2012; Ruan et al. 2012).

The available fossil record of the rails provides only scant information about the origins of the family despite detailed analysis of some species (Olson 1977; Olson 1985; Steadman 1995; Mayr and Smith 2001; Mayr 2005, 2006; Steadman 2006; Mayr 2009). A recent fossil–calibrated analysis of entire mitochondrial genomes of rails and other birds has however estimated their origin in the Eocene around 40.5 (49–33) Million years ago –Mya– (Garcia-R et al. 2014). This has revealed considerable depth of both lineage origin and crown group diversification of ralloids and is at odds with the taxonomic instability that has plagued this group. Deep, phylogenetically distinct lineages would be expected to correlate with stable derived morphological traits.

The deep ancestry and large–scale radiations with high levels of sympatry found in Rallidae provide an opportunity to explore diversification patterns and understand the origin and evolution of biological diversity. I estimate phylogenetic relationships to support taxonomic rationalisation focusing on identification of major clades and species groups and timing of their formation. I establish a temporal phylogeny to provide insights into the underlying mechanisms driving evolution across global rail diversity. I integrate information on prominent morphological, ecological and biogeographical traits

to expand hypotheses about character evolution that may be linked with the presence of rate shifts in diversification.

Materials and Methods

Taxon Sampling

My dataset encompasses ~50% of known extant or recent extinct rail diversity with 70 species in 22 of 33 extant genera. I included at least one representative of every genus comprising more than five described species. The sampling incorporates data from several flightless species including the extinct monotypic *Diaphorapteryx*. My study included partial sequences of the mitochondrial genes cytochrome oxidase *b* (cyt*b*), cytochrome oxidase subunit I (COI) and 16S ribosomal RNA (16S) with a total of ~2900 base pairs (bp). In addition, I sequenced ~1900 bp from fragments of two nuclear genes beta–fibrinogen intron 7 (FGB–7) and Recombination Activating gene 1 (RAG–1) (Supporting material Table S1). Additional, complementary DNA sequences of the same gene fragments were downloaded from GenBank and DNA Barcode of Life (Table S2) to obtain a total of 94 rail species in this study. I mostly follow Taylor (1998) for the initial taxonomic assignment of the species used in this study.

Data collection

Genomic DNA was extracted from fresh tissues using either standard phenol–chloroform methods or the Qiagen QIAamp tissue kit. Preparations from old tissues (toe pads and bones) were carried out in a dedicated ancient DNA (aDNA) laboratory at Massey University (http://www.massey.ac.nz/~strewick/Text%20Files/DNA%20Toolkit.htm). DNA extractions from toe pad samples obtained from museum skins were performed using the Qiagen QIAmp DNA Minikit following standard procedures for aDNA (Rohland and Hofreiter 2007b; Rohland and Hofreiter 2007a; Shepherd and Lambert 2008), while DNA extractions from bones were carried out using phenol–chloroform after decalcification with EDTA and Proteinase K digestion in Tris–buffered saline. Mitochondrial and nuclear DNA fragments were PCR amplified using various combinations of primers (Table S3). Amplification from aDNA was focused on cyt*b* using a combination of primers designed in the Phoenix lab (BR primers, Table S3). Each product was analysed on an ABI Prism 3730 automated DNA sequencer (Applied Biosystems) using the chemical reaction Big–Dye Terminator v3.1 reagents.

All sequences were edited and aligned using Geneious v6.0.5 (Drummond et al. 2012a). Reading frames in protein coding genes (cyt*b*, COI and RAG–1) were identified using amino acid translation in Geneious v6.0.5 (Drummond et al. 2012a). Ribosomal RNA 16S is a conserved mitochondrial marker but indel mutations are common in variable regions corresponding to loops in the ribosomal RNA structure. Gblocks 0.91b (Castresana 2000) was used to remove ambiguously aligned regions of RNA 16S for all the analyses. The same approach was applied to FGB–7, which presented several indel mutations through the fragment amplified. GenBank accession numbers are found in Table S1.

Phylogenetic analyses

Prior to concatenated analyses, single gene, mitochondrial gene and nuclear gene datasets were inspected for evidence of significant incongruence by comparing preliminary Maximum Likelihood (ML) trees using RAxML and a general time reversible model with gamma distribution (GTR + Γ). The model was estimated in ModelTest v3.7 using the Akaike Information Criterion (Posada and Crandall 1998). I observed no significant conflict among individual phylogenies, and found a similar level of support for clades. I performed all subsequent analyses with concatenated data. A 5–way partition by gene strategy was used for the concatenated analysis. I rooted the Rallidae phylogeny using an outgroup comprising *Heliornis fulica, Psophia crepitans, Aramus guarauna*, and *Grus americana* (Table S2). Maximum Likelihood analyses were implemented in RAxML using a GTR + Γ model with bootstrapping automatically stopped employing the majority rule criterion. Bayesian phylogenetic analyses (BA) were implemented in MrBayes using 3 million generations sampled every 4000th generation, a burn in of 10%, and GTR + Γ+ I model of evolution. RAxML and MrBayes analyses were performed via the CIPRES portal (Miller et al. 2010). Trees were viewed using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) and SplitsTree v4.12.8 (Huson and Bryant 2006).

Morphological phenogram

I constructed a Maximum Likelihood phenogram using available osteological, myological and integumentary characters (Livezey 1998) for a range of extant and

extinct rails. I identified a subset of available data corresponding to ingroup and outgroup species for which I had obtained molecular data. Maximum Likelihood (ML) was implemented in RAxML, which supports multi–state morphological data (Stamatakis 2014), via the CIPRES portal (Miller et al. 2010) and the resulting phenogram was visualized in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Molecular dating and diversification rate

Divergence times were estimated using a Lognormal relaxed Bayesian clock implemented in BEAST v1.7.5 (Drummond et al. 2012b). For calibration constraints I used the basal divergence estimation of Rallidae with Normal distribution of 33–49 Mya (95% range) and the basal split of Grues into Ralloidea (Rallidae and Heliornithidae) with Normal distribution of 59–75 Mya (95% range) based on the work of Garcia–R et al. (2014). These timing estimations were obtained using calibration constraints from a Galloanserae fossil (Benton and Donoghue 2007; Benton et al. 2009) and the stem fossil of Sphenisciformes (Slack et al. 2006) with a dataset comprising mitochondrial genomes of representative Rallidae and other birds. I combined the results of three independent runs of 30 million generations each to ensure Effective Sample Sizes (ESS) above 200. Chains were sampled every 4000th generation and a burn–in of 10% (3 million generations) was used. The tree with the times of divergences and Highest Posterior Density (HPD) intervals was visualized using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Temporal shifts in diversification rates of the empirical phylogeny were visualized with logarithmic lineage–through–time (LTT) plots using the package Likelihood Analysis of Speciation and Extinction Rates (LASER v2.4, Rabosky 2006b; Rabosky 2006a) from the R programming environment. I applied a null model of constant birth and death. Alternative diversification rates were compared to understand the effect of speciation and extinction using the R package TreeSim v1.9 (Ford et al. 2009). I incrementally adjusted the extinction rate of the birth–death process ($a = d/b$ from 0.5 to 0.95) in an attempt to obtain phylogenies with different shapes but similar root age (~40 Mya). The resulting empirical phylogeny of the ingroup was conditioned to the approximate diversity of the family Rallidae to infer mean LTT curves and explore departures from the constant–rate model. I simulated 100 phylogenies to 135 extant

species while incorporating the effect of incomplete taxon sampling (i.e. ~70% is the probability of sampling an extant species).

Trait mapping analysis

I used Mesquite v2.75 (Maddison and Maddison 2011) to infer the ancestral state of frontal shield, body size, flightlessness, habitats and geographic distribution traits across the rail tree and estimate the number of times those characteristics have evolved in different species groups or clades. I based ancestral state reconstructions on the topology of the Maximum Likelihood tree from the RAxML analysis with the outgroup excluded to prevent biasing the reconstruction of the characters in the nodes of the tree. Traits were mapped onto the phylogeny using a Likelihood approach and restrictions of equal probability for all state changes with the Mk1 model. Trait data evaluated for the terminal taxa are listed in Tables S1 and S2 based on Taylor (1998). Ancestral character state reconstruction for the frontal shield (a) was coded as: 0 (absent = without frontal shield), 1 (small = not beyond the height of the eyes), 2 (large = notable and covering part of the head); body size (b): 0 (small = 12–19 cm.), 1 (medium = 18–40 cm.), 2 (large = 38–63 cm.); flightlessness (c): 0 (flightless), 1 (volant); ecology (d): 0 (forest), 1 (wetland), 2 (grassland/shrubland); and area distribution (e): 0 (insular), 1 (continent). Because characters cannot be treated as polymorphic for the Likelihood analysis, those species found in more than one habitat or area distribution category were treated as uncertain. However, analysis using Parsimony allowed polymorphic characters and this approach was used to compare the results of both analyses for these traits.

Results

Phylogeny of rails

Bayesian inference yielded a consensus tree that was topologically congruent with the ML tree, with ML bootstrap support and Bayesian posterior probabilities largely consistent among nodes (Figure 1). However, sequence obtained from GenBank for *Micropygia schomburgkii* (JQ175375) was eliminated from all the analyses due to the conflicting phylogenetic signal shown by networks using SplitsTree v4.12.8 (Huson and Bryant 2006) indicating that it was unreliable (result not shown). All analyses reconstructed the flufftail (*Sarothrura rufa*) and the members of *Canirallus* as a separate group from the rails (see also Sibley and Ahlquist 1990; Slikas et al. 2002; Fain et al. 2007; Hackett et al. 2008) and sister to the sungrebe (*Heliornis fulica*). Separate

analyses of the concatenated data using *Sarothrura rufa*, *Canirallus beankaensis* and *Canirallus kioloides* as the outgroup or with those species excluded (i.e. only the ingroup) showed similar support of the nodes and relationships among the ingroup (results not shown).

I identified numerous well–supported clades although deep phylogenetic structure was not fully resolved. Eight major clades based on the results of ML and BA were distinguished in my molecular analyses (Figure 1). These included phylogenetically distinct groups that support several intergeneric and interspecific relationships not recognized by current taxonomy. A main well–supported split between the "*Rallus*" clade and all others was evident, with an indication of polytomy among those other groups (Figure 1). "*Rallus*" is a large clade that includes several currently monotypic genera (*Dryolimnas, Aramidopsis, Eulabeornis, Habroptila* and *Diaphorapteryx*), the Australasian *Gallirallus, Nesoclopeus* and *Lewinia* and the widespread *Crex* and *Rallus*. The "*Fulica*" clade comprises *Fulica* and species within *Gallinula* and *Porzana.* "*Aramides*" contains species of *Aramides, Amaurolimnas, Pardirallus, Neocrex erythrops* and *Porzana albicollis*. *Porphyrio* and *Rallina* are well resolved monophyletic clades as currently recognized. The core "*Porzana*" clade comprised most (but not all) of the *Porzana* species in my analysis plus *Amaurornis flavirostra* and *A. akool.* Species of *Laterallus* and *Coturnicops* along with *Porzana flaviventer, P. spiloptera* and *Anurolimnas viridis* form part of the "*Laterallus*" clade. An additional loose clade comprises the monotypic genera *Gallicrex, Megacrex* and *Himantornis* and two species of *Amaurornis* (*A. phoenicurus* and *A. moluccana*).

The morphological phenogram bears little resemblance to the molecular phylogenetic hypothesis and highlights the historic taxonomic problems (Figure 2). These analyses were concordant only in clustering the species of the *Porphyrio* clade, the close affinity between *Fulica* and *Gallinula*. They clearly demonstrate the present *Porzana* to be polyphyletic. It is evident from the morphological phenogram the grouping of "long–billed" rails (*Rallus, Gallirallus, Dryolimnas* and *Pardirallus*), and the inclusion of *Sarothrura* and *Canirallus* within the core Rallidae contrary to molecular analyses.

Figure 1. Maximum Likelihood tree based on a 5–gene concatenated analysis. Numbers at nodes indicate bootstrap supports (bs) over 70% and posterior probabilities (pp) over 0.90 are indicated in each brach.

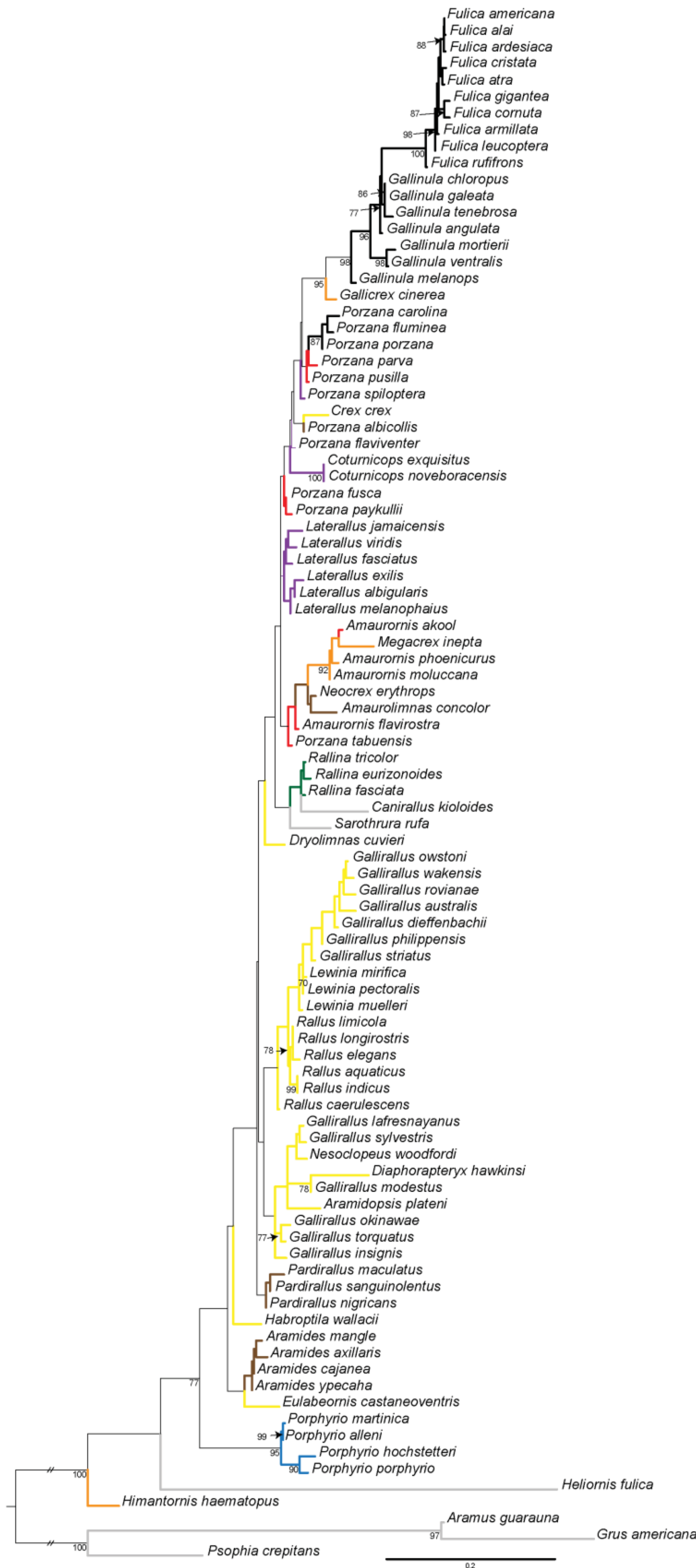


Figure 2. Maximum Likelihood tree based on 570 osteological, myological and integumentary characters compiled by Livezey (1998) for species used in molecular analyses. Bootstrap supports over 70% are indicated in the nodes. Branch colours correspond to species in major clades based on my molecular results. Species in gray are of the outgroup.

Timing and rate of diversification

My results suggest that major clades "*Laterallus*", "*Fulica*", "*Rallus*" and "*Gallicrex*" originated around the Oligocene/Miocene boundary (Figure 3). However, most of the cladogenesis in "*Fulica*" and "*Rallus*" has been much more recent and with the currently recognized *Fulica* and *Gallirallus* groups. Around 13 (16–10) Mya marks the split between the coots (*Fulica*) from gallinules or moorhens (*Gallinula*). Diversification of the "*Rallus*" clade started in the Oligocene/Miocene boundary around 23 (29–19) Mya. The split of the *Gallirallus* group from other "*Rallus*" (*Dryolimnas, Crex, Aramidopsis, Lewinia* and *Gallirallus striatus*) occurred 21 (26–17) Mya. A first split in the *Gallirallus* group separates the small flightless Halmahera species *Habroptila wallacii*, and the giant flightless Chatham *Diaphorapteryx hawkinsi* from all other *Gallirallus* species around 14 (18–10) Mya. Lineage formation and diversification in "*Aramides*", "*Porzana*", *Rallina* and *Porphyrio* occurred in early and Mid–Miocene time. The origin of the *Rallina* clade is around 19 (24–14) Mya, "*Aramides*" and "*Porzana*" clades originated about 18 (22–14) Mya, and *Porphyrio* is the youngest about 15 (20–11) Mya.

The lineage accumulation analysis (Figure 4) favoured a near constant rate of lineage increase in Rallidae, although a cluster of short internal nodes dated to between 40 and 30 Mya indicate a period of relatively abrupt diversification. Some acceleration is also apparent during the Early Miocene (~ 18 Mya) and further rallid diversification during the Pliocene (~ 6 Mya) is mainly associated with cladogenesis within *Gallirallus* and *Fulica*, as currently used (Figure 4). The best combination of parameter values for the LTT plots of simulated phylogenies (Figure S2), resulting in basal divergence close to the root age of Rallidae and producing an upturn in the number of lineages toward the present, was achieved with slow growth rate (0.4) and relative high *b*/*d* ratio (0.75).

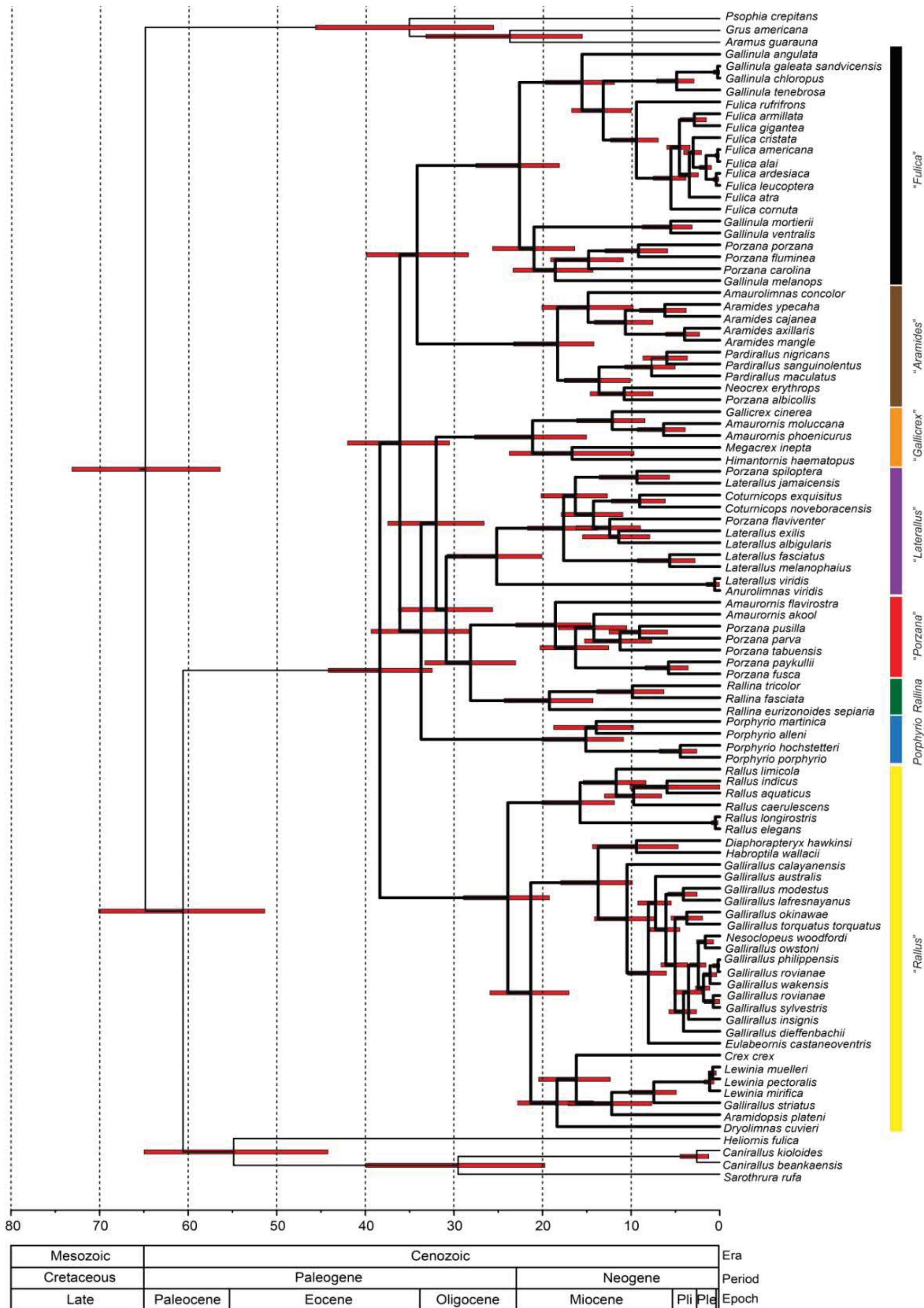


Figure 3. Evolutionary time tree of the family Rallidae. Chronogram based on a relaxed–clock model calibrated with timing of split of Grues into Ralloidea (59–75 Mya) and crown Rallidae (33–49 Mya) estimated in Chapter 1. For each node the estimated time of divergence is indicated with a bar representing the 95% HPD intervals of node ages. The time scale is in millions of years ago (Mya) and geological eras, periods and epochs are indicated where Pli, Pliocene and Ple, Pleistocene.

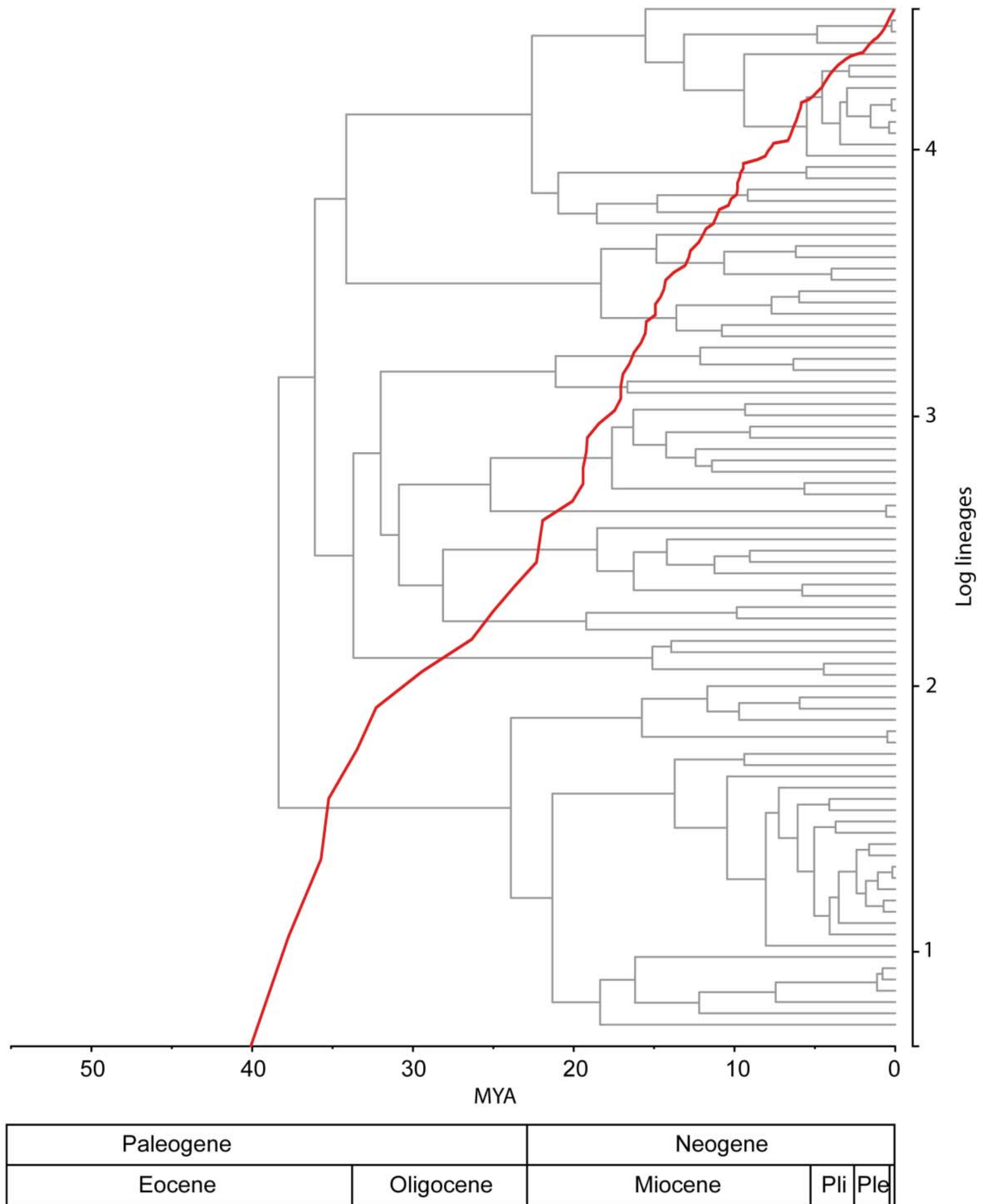


Figure 4. Lineage Through Time plot representing the number of lineages (ln) in the Rallidae radiation under a constant–rate model of diversification. The red line represents the average of the number of lineages using the ML analysis of speciation in LASER.

Trait mapping

The frontal shield, which is a prominent feature of some species, appears to have evolved late in ralloid history (about 15 Mya), and independently in four main lineages within "*Fulica*", "*Gallicrex*" and *Porphyrio* clades (Figure 5a). The two currently recognized genera with the largest frontal shields are *Porphyrio* and *Fulica* (within the

current *Porphyrio* and "*Fulica*" clades, respectively). This trait represents independent evolution suggesting morphological convergence through similar selection. My analysis showed that this trait might be plesiomorphic in *Porphyrio* clade. Present day lineages with small frontal shields are relatively younger (< 10 Mya) than those with large frontal shields. The ancestral character state in groups with small frontal shields is equivocal.

The parsimonious evolutionary scenario for body size suggests that small size has evolved twice and large size four times (Figure 5b). Large species are found in volant and flightless lineages that otherwise also include medium size species, whilst small birds are mostly confined to "*Laterallus*" and "*Porzana*". The "*Fulica*" clade is inferred as having become larger from a medium size ancestor around 10 Mya and the *Gallirallus* lineage appears to have reverted around 6 Mya from a larger ancestral state to a medium size. Likely this is an artifact arising from extinction of relatively small ancestral flying species; an inference borne out by analysis of flight across the tree. Instances of flightless species are found through the phylogeny of rails, but the deepest node with an inferred flightless ancestral state in the present analysis is in the "*Rallus*" clade (Figures 5c, 6a).

Likelihood and Parsimony analyses of ancestral habitat yielded no conflicts with the exception of the reconstruction for the group comprising *Lewinia, G. striatus, Aramidopsis, Crex* and *Dryolimnas* (Figures 6b, S3). For the *Gallirallus* lineage the likelihood approach reconstructed as ambiguous the three types of habitats with similar probability (36% for wetland, 36% for grassland and 28% for forest), while uncertainty in the parsimony analysis was among forest and wetland (50% for both types of habitats). Other clades present wetland as plesimorphic (Figure S3). Mapping the evolution of distribution types shows that islands could be construed as ancestral for the *Gallirallus* lineage and the allied group of species including *Lewinia, Gallirallus striatus, Aramidopsis, Crex* and *Dryolimnas* (Figures 6c, S4).

Discussion

Phylogeny and trait evolution of rallids

Maximum Likelihood and Bayesian phylogenetic analyses of my multigene dataset produced congruent topologies with many well–resolved nodes that allow exploration of family wide–patterns of phenotypic diversification. My molecular analyses confirm that *Sarothrura* and *Canirallus*, which were previously placed in Rallidae have affinities outside the family, and are better treated as a distinct family Sarothruridae (Slikas et al. 2002; Fain et al. 2007; Hackett et al. 2008). The phenetic analysis of morphology places these species among true rails, revealing the source of past misleading taxonomy (Figure 2). Within the Rallidae I recognized eight major clades that help resolve taxonomic confusion arising from evolutionary convergence. Some of these clades render current genera as para– or polyphyletic. The molecular phylogenetic treatment of genera therefore reconciles a seemingly arbitrary taxonomic treatment (Table S4) that has developed since Linnaeus (1758), but is in many respects consistent with the simpler earliest proposals. Prominent in the morphological phenogram is the clustering of species that share the traits of flightlessness and large size (Figure 2). Analysis of 570 osteological, myological and integumentary characters (Livezey 1998) grouped, for example, flightless species of the *Gallirallus* group (*Gallirallus sylvestris, G. lafresnayanus* and *Nesoclopeus poecilopterus*) and *Porphyrio* (*Porphyrio hochstetteri, P. mantelli* and *P. kukwiedei*), with the exclusion of their respective flying relatives (Figure 2, and see Figure 3 in Livezey 1998). Here homoplasy among flightless species obscures phylogenetic history (Olson 1973b), and this also affects inferences of ancestral character reconstruction (see below). Similarly, representatives of *Porzana* (*Porzana pusilla, P. carolina, P. porzana* and *P. fluminea*) form a cluster (Figure 4 in Livezey 1998) that is explained by their shared gray underparts, olive and black dorsum streaked with white, and buffy juvenile plumage (Olson 1973a). These species do not form a natural group in molecular analysis. I also found that traditional treatments of *Fulica* include only species with black plumage, large frontal shields and lobed toes, whereas other species with variable plumage, small frontal shield and straight toes (currently in *Gallinula*) also belong in the same clade.

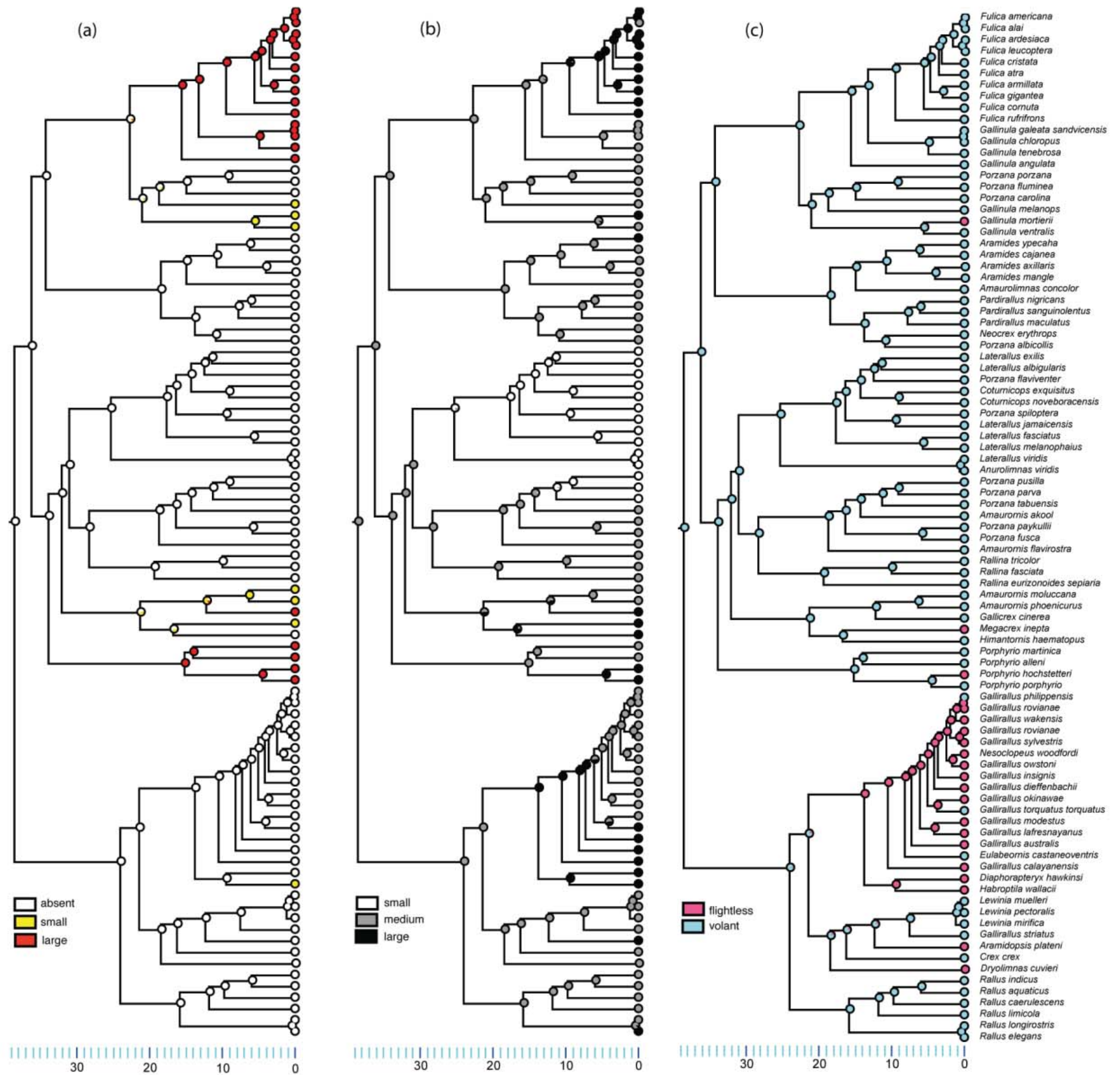


Figure 5. Ancestral state reconstructions based on the Maximum Likelihood tree and using the likelihood model in Mesquite v.2.75 of: frontal shield (a); body size (b); and flighted condition (c). Coloured pie–charts represent proportions generated from the different assigned states of the character (see colour legends).

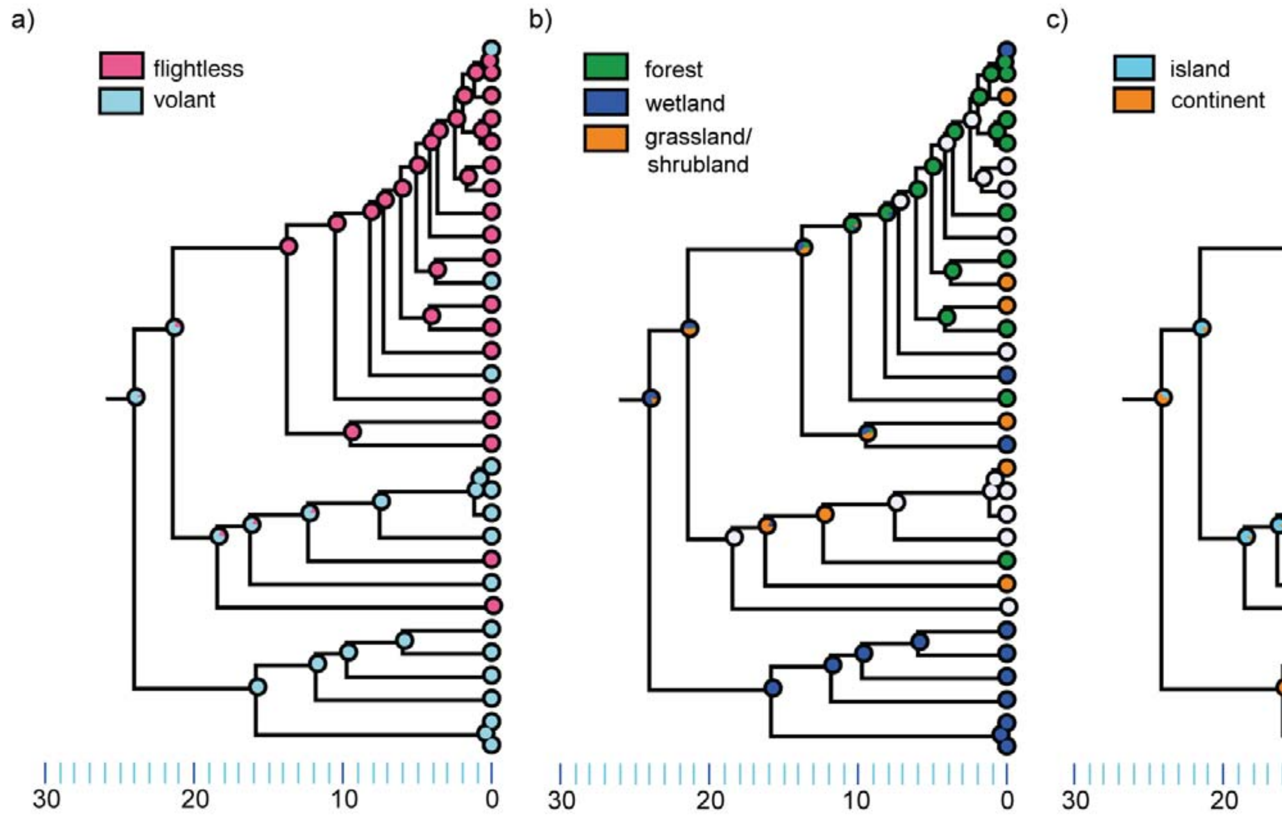


Figure 6. Ancestral state reconstruction based on likelihood analysis of (a) flighted condition, (b) habitat, and (c) distribution
coloured pie charts indicate species with polymorphic states of the character and are coded as uncertain in the data matrix.

Contrary to previous proposals (e.g. Olson 1973a), forest is not supported as the ancestral habitat of the family, instead my results suggest that wetland is the most probable ancestral state for rallid habitat (Figure S3). At the very least, wetland appears to be the predominant condition among extant taxa. My reconstruction also shows that island distribution is a character that has evolved mainly in lineages within "*Rallus*", while continental distribution is the inferred ancestral state for all other groups in Rallidae. This finding mostly reflects the regional importance of *Gallirallus* in the "exploded continent" of Oceania and its recent diversification is mainly linked with isolation and adaptation to novel selective regimes that results in a tendency for enlarged, flightless forest–floor specialists.

The majority of flightlessness species of *Gallirallus* ("*Rallus*" clade) appear to have originated during the Late Miocene through the Pleistocene (Figure 6a). Fitness advantages on oceanic islands that usually lack terrestrial mammal predators favours reversion to a terrestrial lifestyle in a trade–off between energy conservation and predator avoidance (Olson 1973b; McNab 1994; McCall et al. 1998; McNab and Ellis 2006). The incidence of flightless species is so high that ancestral state analysis returns the ambiguous inference that flight has been re–evolved from flightless ancestors. Other evidence suggests this to be highly unlikely (Olson 1973a; Trewick 1997a). There are no authenticated examples of the re–emergence of flight in neoaves and this result is revealing about the sensitivity of ancestral state reconstructions to sampling of tip traits (Cunningham et al. 1998; Omland 1999; Crisp and Cook 2005; Ekman et al. 2008; Crisp et al. 2011). The apparent re–emergence of flight in this clade must reflect directional asymmetry in dispersal of birds of the banded rail *Gallirallus philippensis* lineage; *G. philippensis* is phylogenetically nested amongst flightless *Gallirallus* species. In fact, the flying lineage must have persisted through time and repeatedly established island populations each of which gave rise to insular flightless species that locally replaced their flying founder (Olson 1973b; Trewick 1997a). Likewise, the inference that large size is ancestral in *Gallirallus* is probably correlated with the evolutionary convergence of derived flightless species.

Lineage diversification and biogeography

Dispersal ability and novel ecological opportunities have allowed diversification and retention around the globe of lineage diversity in multiple clades within Rallidae (Olson

1973a; Ripley 1977). Bursts of lineage formation span archipelagos and continents. The result is a biogeographic pattern involving representation of most clades in all habitable continents rather than generic radiations being associated with particular geographic regions (Figure S5). Rapid and shallow speciation is sometimes due to radiation on islands after colonisation (e.g. *Gallirallus*) and habitat patches in continental landscapes (e.g. *Fulica*), but other processes are also implicated. My analysis tracks increasing episodes of diversification over 30 Mya. Old clades are important for rail diversity and diversification may have been related to occupancy of novel habitats propelled by major climatic shifts in the past (Zachos et al. 2001; Böhme 2003; Kürschner et al. 2008; Tolley et al. 2008). The curve of the LTT plot in the empirical phylogeny (Figure 4) showed a pattern of high initial diversification followed by deceleration consistent with density–dependent cladogenesis after available ecological space was filled and constrained by resource limitation (Rabosky and Lovette 2008). This pattern of lineage accumulation is characteristic of a near constant birth–death rate process with a low extinction rate. However, the relatively high *b*/*d* ratio from LTT plots of simulated phylogenies (Figure S2) is consistent with the signature of extinction dynamics registered in different ralloid fossil clades (Olson 1977; Steadman 2006). Such levels of extinction may yield a highly stochastic birth–death process and more species–rich clades (Claramunt 2010).

The molecular phylogeny suggests differing rates of diversification across the family. "*Rallus*", "*Fulica*" and "*Laterallus*" retain most species diversity within Rallidae compared to other clades, and contribute to the accumulation of diversity over time rather than a short–lived burst of speciation. Phenotypic differentiation associated principally with mate recognition (e.g. frontal shield) and morphological innovations (e.g. flightlessness) are also important in lineage diversification (Figures 5, S3 and S4). Frontal shield evolved in four separate lineages within "*Gallicrex*", "*Fulica*" and *Porphyrio* clades around 15 Mya. In "*Fulica*" and *Porphyrio*, frontal shield is known to be associated with social interactions such as status, dominance, mate recognition and choice (Gordon 1951; Craig 1977; Crowley and Magrath 2004; Dey et al. 2014) and this may be the case with other characters (e.g. plumage colour) in other clades that are involved in sexual selection and speciation. Together these features promote speciation of this cosmopolitan family and accumulation of diversity within rallid clades.

Distribution and ecological diversification of rails

Taking into account available information (Trewick 1997a; Slikas et al. 2002; Kirchman 2012; Ruan et al. 2012) and based on the present analyses, a reduced number of genera provides a clearer hypothesis of how diversity within Rallidae is distributed, where each clade may have originated, and possible directions of range expansion (Figure S5). Many currently recognised genera are embedded within widespread clades that are less morphologically homogeneous. Several monotypic genera are strongly supported as belonging to larger widespread clades. In some cases, traits used in taxonomy agree with subclades inferred from molecular data but these are nested among species that share some of the supposedly diagnostic morphological features (e.g. *Fulica* within "*Fulica*"). In other cases, species that share morphological traits are clearly shown by molecular analysis to be non-monophyletic groups resulting from convergence (e.g. *Porzana*).

The large "*Rallus*" clade has a worldwide distribution but may have originated in the Americas. *Rallus* is traditionally applied to the subclade of northern hemisphere species, with *Gallirallus* used for species around Oceania. However, this has not always been the case and at one stage *Gallirallus* was applied to only some of the flightless island endemics of this group (Olson 1973b). Other genera have in the past been erected to deal with supposedly abberant island species including *Ocydromus* (= *Gallirallus australis*) in New Zealand and *Cabalus* (= *Gallirallus modestus*) on Chatham island. Even today some island species that fall within this clade are classified in additional genera (e.g. *Nesoclopeus* in Fiji). A second extinct species on the Chathams islands in the "*Rallus*" clade is currently placed in the monotypic *Diaphorapteryx*, and a third extinct Chatham "*Rallus*" species (*G. deiffenbachii*) has sometimes been considered to be a subspecies of the widespread volant *G. philippensis* despite significant size difference and little likelihood that it could fly (Ripley 1977; Trewick 1997b). In addition, the monotypic genera *Atlantisia* (Innaccessible island) and *Rougetius* (east of Africa) could be included within the "*Rallus*" clade (Olson 1973a).

Gallirallus australis (weka) is at the tip of a long phylogenetic branch that might, parsimoniously, be inferred as implying existence of this flightless form in New Zealand since Miocene time. Alternatively, *G. australis* may be the remains of a subclade that included flying relatives that are now extinct. It is not possible to

determine where on the long branch leading to *G. australis* flightlessness evolved, but the evolutionary convergence of morphologically similar endemic flightless species on other Pacific islands shows that it is not necessary to assume *G. australis* evolved a long time in the past. In contrast, *Eulabeornis castaneoventris* (chestnut rail) of north Australian mangrove also sits on a long phylogenetic branch in “*Rallus*” but has retained flight. The Eurasian landrail or corncrake *Crex crex* has long been treated as ecologically and thus taxonomically distinct but it falls within the “*Rallus*” clade and shares many plumage and morphological features with typical rails in this group. Indeed Linnaeus (1758) originally placed *Crex crex* in *Rallus*.

Black *Fulica* coots are readily recognised and this is one of the genera established by Linnaeus (1758). *Fulica* coots share the very distinctive trait of pale grey flatten toes that render them excellent swimmers. This is a synapomorphy that is demonstrated by the clustering of these species in molecular analysis but is of relatively recent origin. The emergence of *F. rufifrons* from the base of the *Fulica* lineage is consistent with the presence of distinctly narrower lateral membranes of the toes in this species compared to other coots (Olson 1973a). Their normal habitats are lakes and slow moving rivers (Taylor 1998) and the patchiness of these conditions demands that coots are strong fliers. Indeed the global distribution of this group of birds shows that ancestors must have moved among continents; one species is endemic to Hawaii (*F. alai*) but closed related to the North and Central American *F. americana*, while another (*F. cristata*) is centred in Africa. Four other species have sympatric ranges in South America but show marked differences in size and distinctive frontal shield colours and ornaments that likely aid mate recognition. One species (*F. atra*) occurs through Europe and Asia and appears to have extended its range in recent times into Australasia. Island colonisation resulted in the former existence of large endemic species including *F. newtonii* on the Mascerene Islands in the Indian Ocean, *F. prisca* in New Zealand and *F. chathamensis* on the Chatham Island.

Fulica is nested among species currently assigned to *Gallinula* rendering the latter paraphyletic. In fact *Gallinula* falls into two sister clades, one with *Fulica* and the other with species of *Porzana* (*P. carolina, P. fluminea* and *P. porzana*). Despite their current generic distinction, species in this subclade have broadly similar plumage and habits. Their chief distinction being that plumage is overall less dark than the coots and other

Gallinula (e.g. the moorhen *G. chloropus* which Linnaeus (1758) originally placed in *Fulica*). On a relatively long branch from the base of the "*Fulica*" clade are the pair of relatively large, dark Australian gallinules, *G. ventralis* and the flightless Tasmanian *G. mortierii* (formerly *Tribonyx*). The frontal shield is variously developed in "*Fulica*" being large and red in *Gallinula galeata*, large and white in *Fulica atra*. In *F. cristata* the white shield is embellished with red knobs and in *F. cornuta* the shield is black and wattle-like. Most *Gallinula* and *Porzana* in this clade have much smaller, sometimes nondescript pale shields.

"*Porzana*" is widespread across Europe, Africa, Asia and Oceania, even when many of the species usually ascribed to this genus (*P. porzana, P. carolina, P. fluminea, P. albicollis, P. flaviventer* and *P. spiloptera*) are excluded for phylogenetic reasons. True "*Porzana*", which are typically inhabitants of swamps and reed beds, includes species in continental areas and some islands but I note some must be recent arrivals. For example, New Zealand, Australia and other Pacific islands share *P. tabuensis*. Several island endemics in this group (*P. palmeri* in Laysan island, *P. monasa* in Kosrae island, *P. atra* in Henderson island and *P. sandwichensis* in Hawaii) are descended from volant widespread species (Slikas et al. 2002).

"*Laterallus*" is largely confined to the Americas and includes the New World *Porzana* (*P. spiloptera* and *P. flaviventer*) as well as traditional *Laterallus, Anurolimnas* and *Coturnicops*. The latter includes *C. exquisitus* outside the Americas in Asia. The monotypic *Micropygia* is also endemic to the New World and probably belongs to the "*Laterallus*" clade (Howard and Moore 1991). "*Aramides*" is another New World group that includes *Aramides, Pardirallus,* the monotypic *Amaurolimnas concolor* and the American *Porzana albicollis* and *Neocrex erythrops*. *Cyanolimnas* might also be embedded within "*Aramides*" sharing finely barred flanks, buffy crissum, drab olive-brown above and dark gray below, usually with light throats, bill shape (with a paint-like red spot at the base) and frontal plate (Olson 1973a). I note that these two New World clades ("*Laterallus*" and "*Aramides*") are not phylogenetic sisters.

Rallina is found across Asia and Oceania and probably includes the three currently recognized species within *Gymnocrex* (Olson 1973a) which are endemic to islands in Southeast Asia. The "*Gallicrex*" clade includes the Asian monotypic *Gallicrex cinerea,*

two *Amaurornis* species from Asia and Oceania (*A. moluccana* and *A. phoenicurus*), and the monotypic African Nkulengu rail *Himantornis haematopus* that is allied to another aberrant species, the New Guinea flightless rail *Megacrex inepta*. *Himantornis haematopus* has been considered the most "primitive" rallid and consequently traditionally placed in its own subfamily, Himantornithinae (Olson 1973a; Ripley 1977; Livezey 1998). My results strongly indicate the placement of *H. haematopus* within core rails (i.e. discard subfamilies). Supposedly "primitive" characters in *H. haematopus* (Olson 1973a; Livezey 1998) are more parsimoniously inferred as uniquely derived, perhaps showing evolutionary response to habitat conditions. Species in this group inhabit a wide range of habitats including swamps and forests. An African origin might be ascribed to *Porphyrio* even though this requires long distance dispersal into the Americas and Europe, Asia and Oceania (Figure S5). Like "*Rallus*" and "*Porzana*", *Porphyrio* includes many examples of island colonisation and instances of endemism involving evolution of flightlessness (e.g. *P. hochstetteri* in New Zealand).

Phylogenetic evidence suggests that diversification patterns within the Rallidae are structured ecologically and geographically. Widespread clades reflect the fact that long–distance dispersal has overcome potential ecological barriers including expanses of open water and land. I note, however, that representation of each clade around the world is often by one or few supertramp species such as purple swamphen (*Porphyrio porphyrio*) within *Porphyrio*, banded rail (*Gallirallus philippensis*) within "*Rallus*", common coot (*Fulica atra*) and common moorhen (*Gallinula chloropus*) within "*Fulica*", and Baillon's crake (*Porzana pusilla*) and spotless crake (*Porzana tabuensis*) within "*Porzana*". These species are widespread across several thousands of kilometres often with high numerical abundance in local populations (Diamond 1982; Mayr and Diamond 2001; Sullivan et al. 2009; BirdLife International 2014). Dispersal behaviour as an important life–history trait in these species that has fueled a rapid radiation in archipelagos, particularly in the south west Pacific (Diamond 1977; Trewick 1996, 1997a; Trewick 1997b; Mayr and Diamond 2001). High dispersal ability, huge abundance and rapid adaptation of species (Olson 1973a, b; Ripley 1977; Ripley and Beehler 1985; Kirchman 2009), has resulted in speciose and widespread genera (Darwin 1859). The occurrence of species with remarkably different ecology and dispersal ability in the same clade likely indicates rapid shifts in these attributes over evolutionary time (Andersen et al. 2014).

Evolution in the Rallidae includes features that have been observed separately in other bird family radiations. The babblers (Moyle et al. 2012) appear to have a younger history than the rails and shows elements of dispersal and speciation around the world, but the majority of extant taxa are in Asia and the ancestral condition is inferred as Asian. Within the babblers, white–eyes (Moyle et al. 2009) show regional radiations but with spatial overlap of clades because one lineage has been more successful in spreading geographically. In contrast, New Zealand (moa, Bunce et al. 2009), Hawaiian honeycreepers (honeycreepers, Lerner et al. 2011), and Madagascan vangas (vangas, Jønsson et al. 2012) show rapid diversification, but only within geographically constrained island settings. In a more expanded geographical setting but more recently radiated in comparison to rails, passerine honeyeaters (Driskell and Christidis 2004; Andersen et al. 2014), ovenbird–woodcreepers (Irestedt et al. 2009; Claramunt 2010; Derryberry et al. 2011), whistlers (Jønsson et al. 2010), and non–passerine auks (Weir and Mursleen 2013) show patterns of lineage accumulation involving specialisation of particular morphological characteristics (e.g. cranial kinesis, plumage, bill) and behaviour associated with ecological specialisation. Rails show resilience in diverse conditions and can be found in different habitats/continents because niche partitioning (space and time) is linked to adaptation of organismal traits (structural or behavioural). Morphological divergences and convergence have obscured the relationships and biogeographic patterns within Rallidae, especially among endemic island taxa or archipelago genera and extended to continental relatives. This corresponds to a spatial and temporal dependency of historical rail diversification with increasing rates focusing mainly at species–level in different geographical scales.

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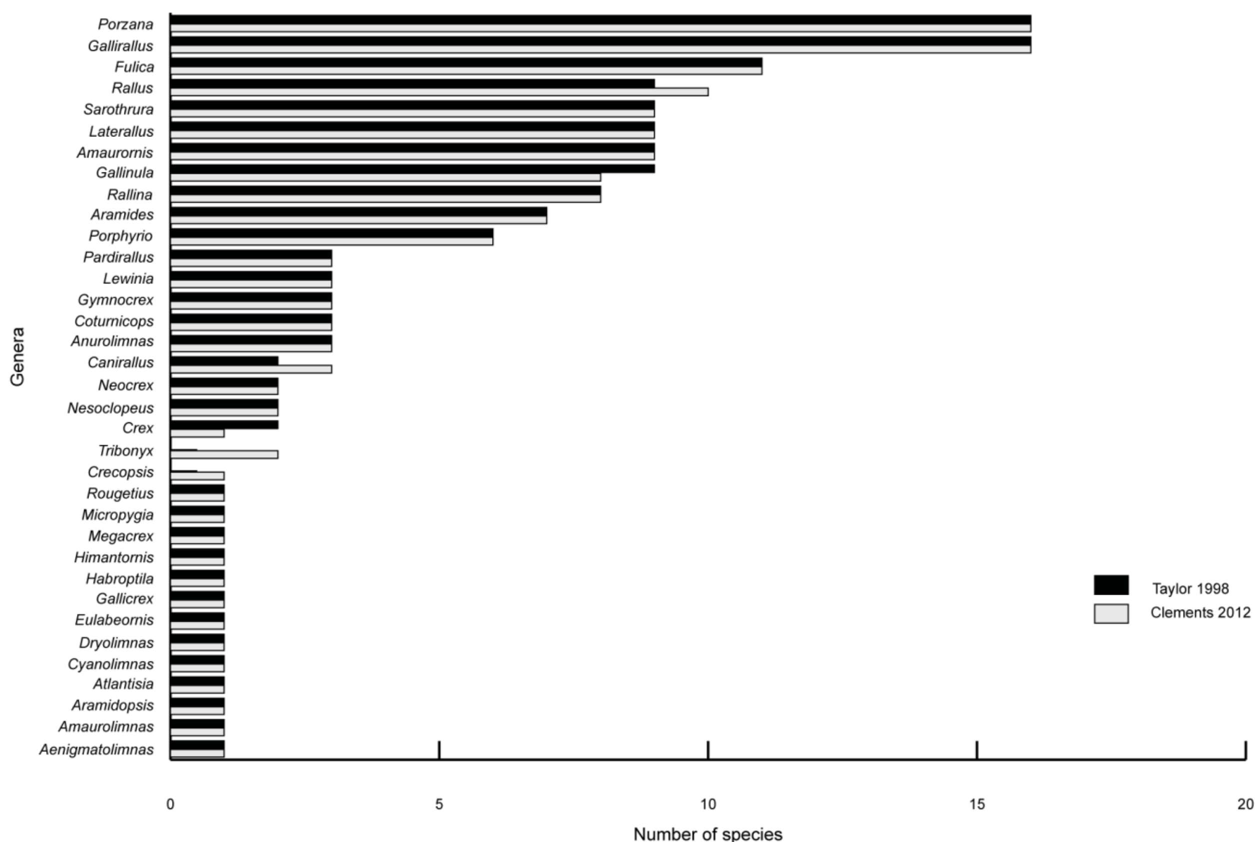
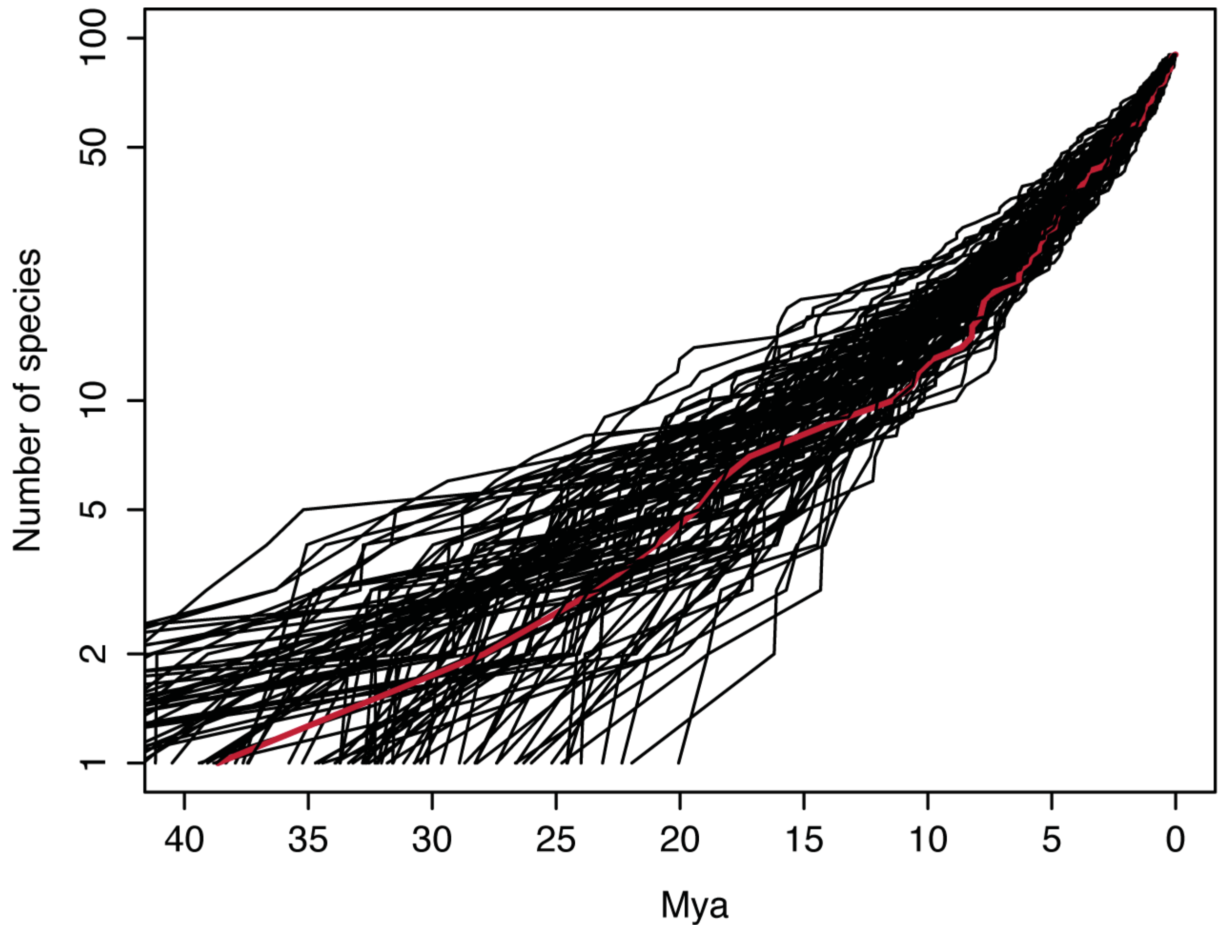
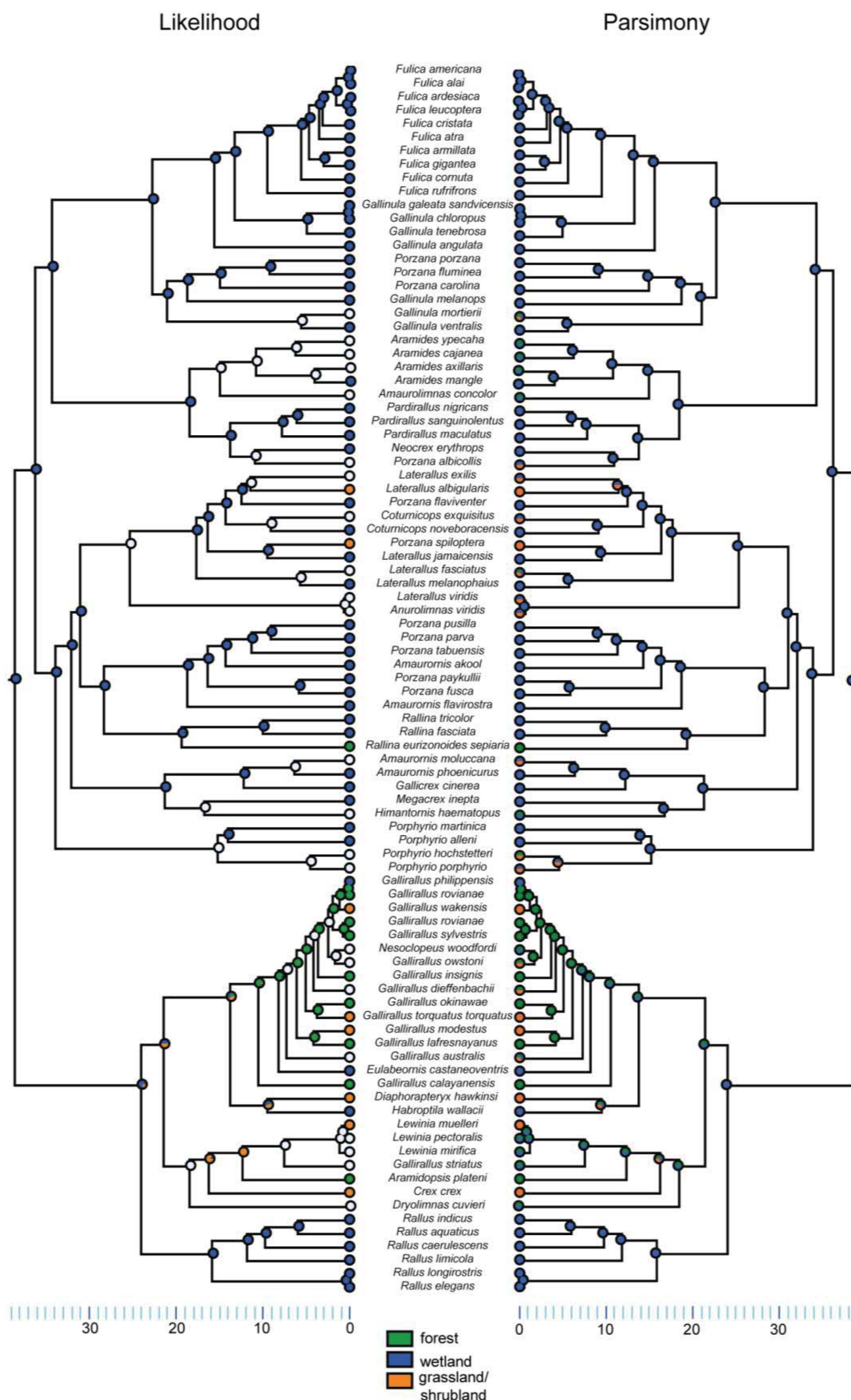
Supplementary Information–Figure S1. Summary of extant and recently extinct taxonomic diversity in the most up to date treatments of Rallidae following Olson (1973a). There remain some differences in the number of genera and species among these classifications because new taxa have been genetically verified (e.g. *Rallus indicus, Gallinula galeata* and *Canirallus beankaensis*; see Groenenberg et al. 2008, Tavares et al. 2010 and Goodman et al. 2011) and a different treatment of one species within *Crex* (*C. egregia* placed in the genus *Crecopsis*) and two species within *Gallinula* (*G. ventralis* and *G. mortierii* placed in *Tribonyx*) in the Clements' list (2012). Other taxonomic treatments can be found in Sharpe (1894), Peters (1934), Berndt and Meise (1960), Fisher and Peterson (1964) and Ripley (1977). For a summary of taxonomic history of rallids see Taylor (1998) and Livezey (1998).



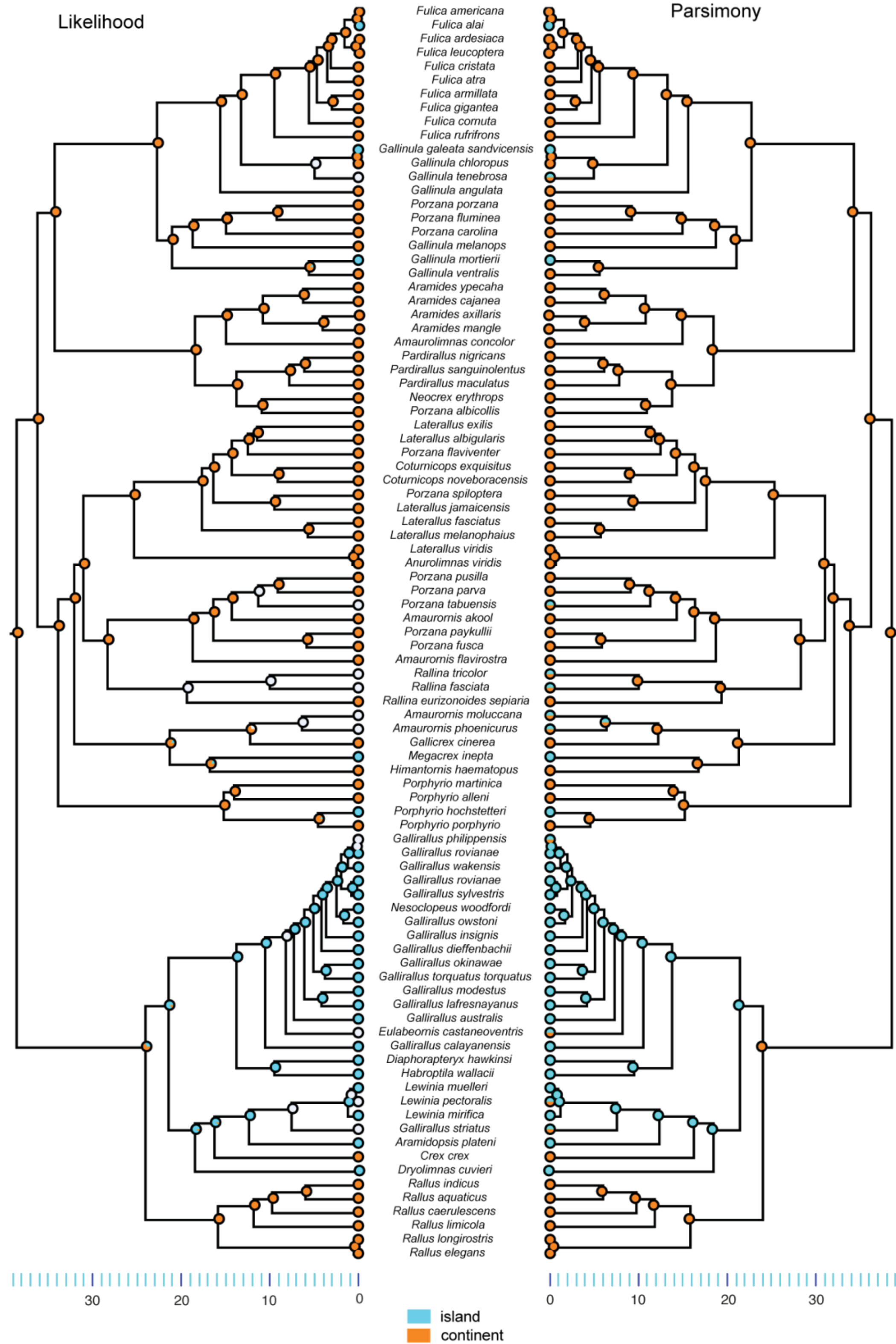
Supplementary Information–Figure S2. Expected LTT plot from 100 simulated phylogenies allowing incomplete sampling under the constant rate birth–death process. The red line represents the average of the expected number of lineages for the Rallidae crown group plotted against LTT curves of 100 phylogenies (black lines) using the package TreeSim. The LTT plots were simulated under a 4:3 birth–death process of the present rail diversity to produce a tree with the same number of species and basal divergence as the observed phylogeny.



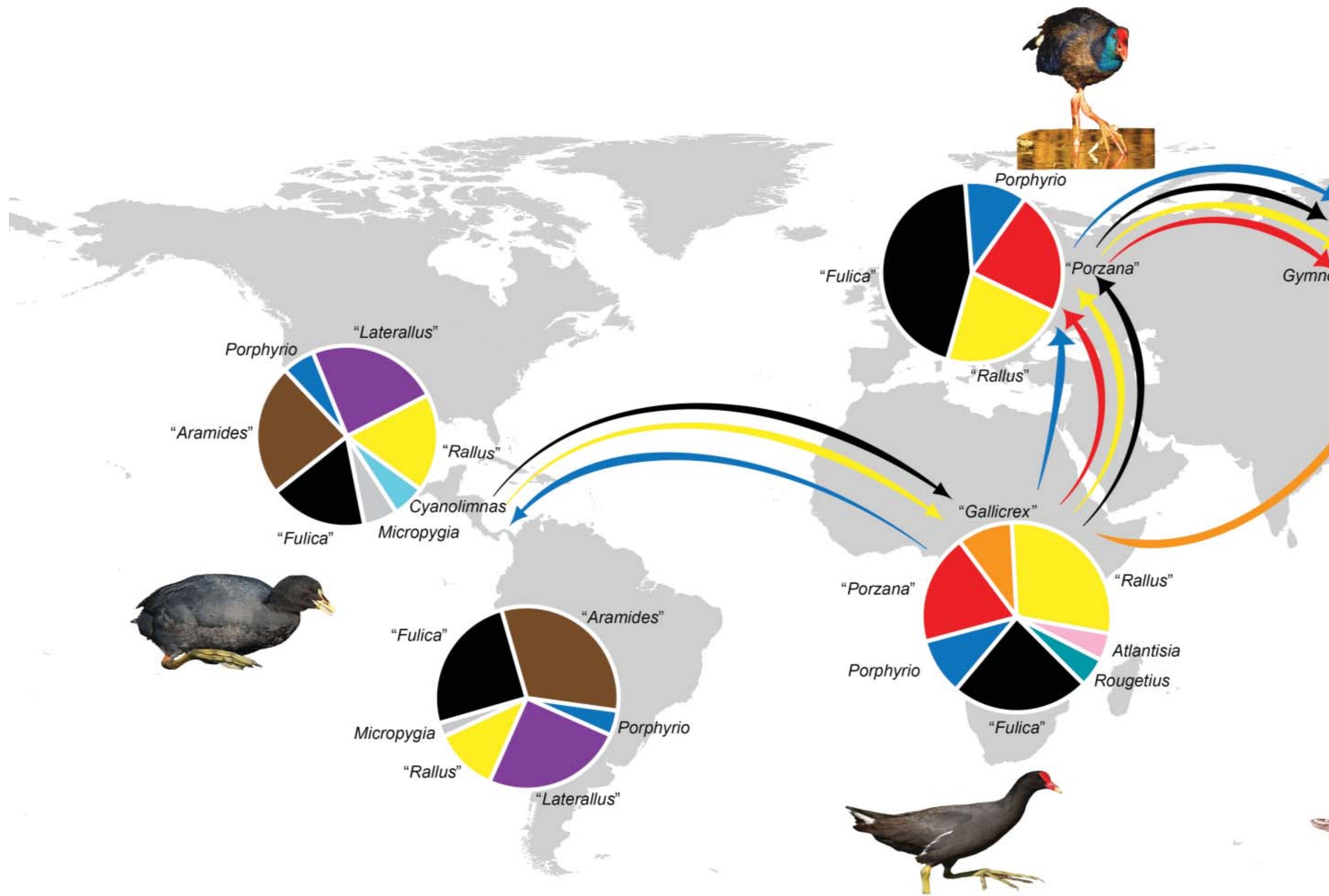
Supplementary Information–Figure S3. Ancestral state reconstructions for habitat based on likelihood (left cladogram) and parsimony (right cladogram) models. Terminals without coloured pie charts in the likelihood analysis indicate species with polymorphic states of the character and are coded as uncertain in the data matrix.



Supplementary Information–Figure S4. Ancestral state reconstructions for area distribution based on likelihood (left cladogram) and parsimony (right cladogram) models. Terminals without coloured pie charts in the likelihood analysis indicate species with polymorphic states of the character and are coded as uncertain in the data matrix.



Supplementary Information–Figure S5. Proportional diversity of clades in Rallidae by geographic regions (continent/subcontinent) and hypothesized direction of dispersal (indicated by arrows) based on the molecular phylogeny obtained here and previous studies. Proposed clades common to more than one region are indicated by identical colour in each pie chart as on Figure 1. I follow several assumptions to recognize and reorganize the diversity within this family of birds according to available information. "*Fulica*" clade is composed of *Fulica, Gallinula* and *Porzana* species closely related in my tree and the missing species in my sampling within *Fulica* and *Gallinula*. "*Aramides*" clade is grouped by species in this genus, the monotypic genus *Amaurolimnas, Pardirallus* species, *Neocrex erythrops* and *Porzana albicollis* plus *Aramides* species and *Neocrex colombiana* which are not included in my sampling. "*Porzana*" is integrated by the group of *Amaurornis* and *Porzana* species plus the *Amaurornis* and *Porzana* species missing in my sampling that are grouping together with those species in Clade 3 of Slikas et al. (2002). "*Laterallus*" is represented by the species within this genus plus *Anurolimnas, Coturnicops* and *Porzana* species clustering on it and *Laterallus, Anurolimnas* and *Coturnicops* species not included here. "*Gallicrex*" is composed of *Gallicrex, Amaurornis, Megacrex* and *Himantornis* species plus *A. isabellinus*, *A. olivacea* (see Clade 1 in Slikas et al. 2002) and *A. magnirostris* (which are geographically closer to the *Amaurornis* species in my tree), and *Porzana cinerea* and the monotypic genus *Aenigmatolimnas* according to Slikas et al. (2002) (but caution is needed for this because low node support of those latter species within this group). "*Rallus*" clade included *Gallirallus, Eulabeornis, Nesoclopeus, Habroptila, Diaphorapteryx, Dryolimnas, Crex, Aramidopsis, Lewinia* and *Rallus* and the species within *Rallus, Gallirallus, Nesoclopeus* and *Crex* which are not included in my tree. *Porphyrio* and *Rallina* are separately composed by species currently recognized in those genera. The genus *Gymnocrex* and the monotypic genera *Atlantisia, Cyanolimnas, Micropygia, Rougetius* that are not included in my analysis must fall inside of one of the groups suggested here but because I currently have no relevant information they stay as they currently are known. Note that I also do not include within these groups the several extinct species described in Steadman (2006) or previous works (see e.g. Olson 1977) among others (e.g. Mayr 2006). *Sarothrura* and *Canirallus* are not included here (see Results and Discussion). Images courtesy of Ramon Moller Jensen, Edward Smith and Vicente Moreno.


Porphyrio
"Fulica"
"Porzana"
"Rallus"
Porphyrio
"Laterallus"
"Aramides"
"Rallus"
Cyanolimnas
Micropygia
"Fulica"
"Gallicrex"
"Porzana"
"Rallus"
Atlantisia
Porphyrio
Rougetius
"Fulica"
"Fulica"
"Aramides"
Micropygia
Porphyrio
"Rallus"
"Laterallus"

Supplementary information for Chapter 2 can be found on the enclosed CD

Supplementary Information–Table S1. Taxa, voucher numbers, specimen sampling locality, type of tissue used for DNA extraction, ecological, morphological and spatial characters, and GenBank accession numbers. Acronyms for museums are: AMNH = American Museum of Natural History, USA; ANWC = Australian National Wildlife Collection, Australia; BMNH = British Museum of Natural History (Tring Museum), England; EBU = Evolutionary Biology Unit at the Australian Museum, Australia; FMNH = Field Museum of Natural History, USA; LIV = Liverpool Museum, England; LSUMZ = Louisiana State University Museum of Zoology, USA; MPEG = Museu Paraense Emilio Goeldi (Goeldi Museum), Brazil; MZUSP = Museu de Zoologia, Universidade de São Paulo (USP Museum), Brazil; NMNZ = National Museum of New Zealand (Te Papa Museum), New Zealand; NMP = Philippines National Museum, The Philippines; UWBM = University of Washington Burke Museum, USA; ZMUC = Zoological Museum, University of Copenhagen, Denmark. N/A = data not available. An asterisk (*) in the distribution column indicates that those species have an ambiguous classification for this character because they are found to approximately the same extent on continents and islands. A cross symbol (┼) indicate extinct species.

Supplementary Information–Table S2. Taxa, characters, GenBank accession numbers or Barcode of Life sequence ID, and original source of data for additional DNA sequences included in this study. N/A indicates category of information does not apply to the sample.

Supplementary Information–Table S3. Primers for PCR and DNA sequencing of three mitocondrial and two nuclear genes employed in this study. Primers denoted with * were taken from the primer database of the Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North and were designed, modified and/or improved by various researchers.

Supplementary Information–Table S4. Current names of genera and species showing original names, authorities and type species. For different treatments through time see Ripley (1977). The clade division is based in my results. An asterisk (*) showed taxa missing in my sampling. Inclusion and total number of species within each clade are related to Figure S5.

Dispersal and speciation in purple swamphens (Rallidae: *Porphyrio*)[1]

[1] In press: Garcia-R JC, Trewick SA. The Auk.

Abstract

Dispersal, when it is accompanied by reduced gene flow and natural selection, influences speciation rates among groups of organisms. Here, I use molecular phylogenetics, divergence time estimates and population genetics to reconstruct the mode, pattern and tempo of diversification within the wide–ranging purple swamphens (genus *Porphyrio*) with emphasis on the supertramp *Porphyrio porphyrio*. My results suggest that the *Porphyrio* clade arose during the Mid–Miocene in Africa, with a single colonization to the Americas and several other colonizations into Southeast Asia and the Indo-Pacific around 10 Mya. I find that the widespread species *Porphyrio porphyrio* is not monophyletic. Indeed, several subspecies and subspecies groups may represent species level lineages. The *P. p. melanotus* lineage probably reached Australasia during the Pleistocene (600 kya), although some islands were colonised only in the last few hundred years. New Zealand, and some other islands, had previously been colonized (~2.5 Mya) by flying *Porphyrio* that evolved into flightless endemic species. Early and recent lineages are now sympatric. Widespread occupation of oceanic islands implies high dispersal and colonization rates but gene flow probably occurs episodically and following varying routes at different times. This pattern of colonization enables populations to differentiate and ultimately speciate.

Keywords: phylogeography, dispersal, *Porphyrio*, phylogeny, speciation

Introduction

The ability to disperse and colonize new habitats provides organisms with ecological opportunities to harvest novel resources and establish new populations. Vagrant species that reach new regions or isolated islands thousands of kilometres from their traditional breeding range via long–distance dispersal (LDD) may generate new flocks of dispersers before being totally or partially displaced by more efficient competitors (Diamond 1974, 1975). This supertramp strategy can also give rise to numerous phenotypically distinct variants, which may lead to speciation (Simpson 1953; Diamond 1974; Diamond et al. 1976; Grant 1986 ; Whittaker 1998; Crisp et al. 2011). However, for a single species to persist without differentiation over a large, habitat–fragmented range, individuals must move between habitat patches at a rate sufficient to counter the evolutionary effects of isolation. This requirement predicts that such widespread species have a high level of gene flow among populations distributed across several landscapes. Alternatively, relatively small isolated populations can segregate and accumulate phenotypic differences if range expansion is not constant through time or the direction of dispersal changes. Outcomes may vary depending on the number of independent radiations, differences in diversification rates, rate and pattern of gene flow or rapidity of species radiation following a wave of dispersal (Mayr and Diamond 2001; Moyle et al. 2009; Cibois et al. 2011).

Inferring the geographic origin and temporal diversification of organisms is an essential part of biogeography and depends on an accurate estimate of evolutionary relationships among species (Rosen 1978; Filardi and Moyle 2005). In the case of birds, as a result of their capacity to fly and ability to disperse long distances the spatio–temporal patterns of diversification can be challenging, especially when shallow radiations on islands generate differential morphological traits that obscure evolutionary affinities (Filardi and Moyle 2005; Irestedt et al. 2013). Dispersal and adaptation together are important drivers of insular diversification of many bird groups (Pratt 2005; Grant and Grant 2008) and account for much of the diversity that we find today in archipelagos (Trewick and Gibb 2010; Trewick 2011). Colonization of islands sometimes involves loss of ability for further long distance dispersal when flight is not integral to foraging, social interaction or predator avoidance (McNab 1994; McNab and Ellis 2006; Steadman

2006). Reduction and even loss of flight capacity can be an adaptive response to island life, and some speciation may occur with adaptation of flightlessness as a result of altered selective environments (Milá et al. 2010; Sly et al. 2011; Alonso et al. 2012; Runemark et al. 2012).

The bird family Rallidae (Aves: Gruiformes) is diverse and cosmopolitan. It includes species that are common and good dispersers, as well as regional and island endemics. Many oceanic islands that were naturally without terrestrial mammal predators appear to have favoured reversion to a terrestrial lifestyle after colonization and speciation by flying ancestors (Ripley 1977; Steadman 2006). This combination of high dispersal and high endemicity associated with the loss of flight makes them interesting subjects for evolutionary analysis. In particular, the large flamboyant purple swamphens (genus *Porphyrio*) demonstrate extraordinary dispersal capabilities with evidence of multiple invasions apparently spaced out in time resulting in divergences of size, colour and other traits (Ripley 1977; Remsen and Parker 1990; Trewick 1996). Seven species of purple swamphens are currently recognised, four of which are/were present in Oceania region (Trewick 1996; Taylor 1998). Principle among these is the widespread supertramp Purple Swamphen *Porphyrio porphyrio* that occurs from Africa and the Mediterranean east to the Pacific (Ripley 1977). This taxon comprises apparently parapatric morphological variants that have at times been classified into ~13 subspecies or species (Figure 1A) (Ripley 1977; Taylor 1998). Although sometimes considered to be reluctant fliers (Craig 1977; Craig and Jamieson 1990), this taxon has nevertheless established populations on many oceanic islands, throughout the Indian and western Pacific Ocean (Mayr 1949; Ripley 1977; Trewick 1997; Trewick 2011). At least two colonizations of New Zealand resulted in the presence of the endemic flightless herbivores North and South Island Takahe (*Porphyrio mantelli* and *P. hochstetteri*) sympatric with flying swamphens (Trewick 1997; Trewick and Worthy 2001). Some island populations and subspecies are known only from fossils that reveal numerous extinctions following colonization of those islands by people (Steadman 1995; Steadman et al. 1999; Steadman 2006). Insular endemics have been recognised as distinct species on New Caledonia and New Zealand (Balouet and Olson 1989; Trewick

and Worthy 2001) and other Pacific islands (Steadman 1988; Kirchman and Steadman 2006).

I use multi–locus DNA sequence data to generate a dated phylogenetic hypothesis of relationships among *Porphyrio* swamphens and to explore the pattern of gene flow among populations of *P. porphyrio*. I address the following questions to gain insights into the biogeographical origin and diversification of these birds: 1) What is the phylogenetic structure of the genus? 2) What is the likely time of diversification, pattern of dispersal and colonization that explains current diversity? 3) Is there support for a single or multiple range expansions? 4) Are regional subspecies of *P. porphyrio* monophyletic, or is there mismatch between clade structure and taxonomy?

Methods

Sampling

To obtain DNA for analysis, I sampled among bones, toe pads, feathers, blood and muscle tissue from specimens of the 7 known species of *Porphyrio* (Figure 1B), including representatives of *P. porphyrio* subspecies from Africa, Europe, Asia and Pacific islands (Table 1; Figure 1A). Additionally, I sampled several populations in New Zealand and Australia separated by ~1500 km of sea and graded terrestrial landscapes to explore gene flow at different spatial scales.

Laboratory techniques

DNA extraction

DNA extractions from bones and toe pads were carried out in a dedicated ancient DNA (aDNA) laboratory at Ecology Group, Massey University (see DNA Toolkit at evolves.massey.ac.nz). DNA extractions from toe pad samples were performed using the Qiagen QiAMP DNA Minikit (Qiagen) following the manufacturer's instructions and standard procedures for ancient DNA (Cooper and Poinar 2000; Rohland and Hofreiter 2007). DNA from bones was extracted using decalcification with EDTA and Proteinase K digestion in Tris–buffered saline followed by purification with phenol–chloroform. DNA from fresh tissues was extracted in a laboratory geographically separated from the aDNA laboratory using either Qiagen Tissue DNeasy kit (Qiagen)

following the manufacturer's instructions or incubation at 55˚C with Proteinase-K and a CTAB buffer (2% Hexadecyl trimethyl ammonium bromide, 100 mM Tris–HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA), followed by a combined phenol/chloroform/isoamyl alcohol (25:24:1) cleanup.

Mitochondrial and nuclear DNA amplification

Two mitochondrial and one nuclear genes were sequenced for population genetic analyses of *P. porphyrio* in Australia and New Zealand: mitochondrial control region (CR) and cytochrome oxidase *b* (cyt *b*), plus a fragment of the nuclear beta–fibrinogen intron 7 (BFG–7). For phylogenetic analysis of *Porphyrio*, parts of two additional mitochondrial genes (ribosomal RNA 12S and 16S) and one nuclear gene (recombination activating gene 1, RAG–1) were amplified from representative specimens of the currently recognized species and subspecies of *P. porphyrio*. Additional sequences were downloaded from GenBank (Table S1). Standard PCR methods with primers listed in Table S2 were used for amplification of nuclear and mitochondrial fragments. Amplification products were purified with QIAquick PCR clean–up kit (Qiagen) or ExoI/SAP digest. For each PCR product both strands were sequenced using Big–Dye Terminator v.3.1 reagents and an ABI 3730XL automated DNA sequencer (Applied Biosystems Inc.). Short sequences (<200 bp) are included in Appendix without GenBank accession numbers.

Phylogeny and divergence times

All sequences were edited, assembled and aligned using Geneious v.6.0.5 (Drummond et al. 2012a) and checked by eye. Ribosomal genes, CR and BFG–7 alignments were conducted using Gblocks v.0.91b (Castresana 2000) and evaluated by eye. Cytochrome oxidase *b* and RAG–1 were checked for the presence of indels and stop codons. Prior to concatenated analyses I performed individual gene tree analysis to detect spurious branch length patterns and evidence of significant incongruence. I built a supermatrix with a 6–way partition by gene 12S, 16S, CR, cyt *b*, BFG–7 and RAG–1 (Wiens 2006; Holland et al. 2007; Wiens and Moen 2008; Johnson et al. 2012). Maximum Likelihood (ML) trees were implemented in RAxML v.8.0.24 via the CIPRES portal (Miller et al. 2010). I used a general time reversible model with gamma distribution (GTR + Γ)

which allowed RAxML to halt bootstrap resampling automatically (bootstopping) once split support values converged (Pattengale et al. 2010). I conducted Bayesian phylogenetic analyses using MrBayes v.3.2.2 as implemented in the CIPRES portal under a GTR + Γ+ I model of evolution. The model was estimated in ModelTest v3.7 using the Akaike Information Criterion (Posada and Crandall 1998). After performing shorter test runs, I conducted three parallel runs of the Metropolis Coupled Monte Carlo Markov Chain (MCMCMC) algorithm for 5 million generations each, sampling one tree with associated parameter values per 5000 generations, and employing three heated chains and one cold chain. Convergence and diagnostics of the Markov process were visualized using Tracer v.1.6 (http://tree.bio.ed.ac.uk/software/tracer/). The first half million generations (10%) were discarded as burn-in. A burn in of 10% gave optimal results and I obtained Effective Sample Sizes (ESS) above 200 for 95% of the parameters. Maximum Likelihood and Bayesian trees were viewed using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and SplitsTree v.4.12.8 (Huson and Bryant 2006). *Amaurornis flavirostra* was used as an outgroup to root the tree.

Divergence times among lineages of *Porphyrio* were estimated using a relaxed Bayesian clock implemented in BEAST v.1.7.5 (Drummond et al. 2012b). For calibration constraints I used the basal divergence estimate of *Porphyrio* with a normal distribution of 11–20 Ma (95% range) and the basal split of *Amaurornis flavirostra* and *Porphyrio* with a normal distribution of 27–35 Ma (95% range) as previously calculated from an analysis using a widely sample dataset of mitochondrial and nuclear genes (Garcia-R et al. 2014). I combined the results of three independent runs of 30 million generations to ensure ESS scores above 200 for 95% of the parameters in each run. Chains were sampled every 4000 generations and a burn–in of 10% (3 million generations) was used. The tree with times of divergence and Highest Posterior Density (HPD) intervals was visualized using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 1. Taxa, museum voucher numbers, locality, type of tissue, and GenBank accession numbers of data included in this study. A
200 bp and included in Appendix 1.

Species of *Porphyrio*[1]	Subspecies[1]	Museum voucher[2, 3]	Locality[4]	Tissue type	GenBank accession number Mitochondrial genes		
					12S	16S	Cyt *b*
alleni		UWBM 86785	Captive	Muscle	KJ685955		
flavirostris		USNM 623070	Guyana	Muscle	KJ685950	KJ685965	KJ686071
mantelli		NMNZ DM7930	New Zealand	Bone			*
martinica		AMNH DOT7585	USA	Muscle	KJ685956		
albus		NMW 50.761	Lord Howe island	Toe pad			KJ686084
porphyrio	*bellus*	WAM 36186	WA, Australia	Muscle	KJ685953	KJ685960	KJ686067
		WAM 34492	WA, Australia	Muscle			KJ686082
		WAM 27444	WA, Australia	Muscle			KJ686064
		ANWC 50436	WA, Australia	Muscle			KJ686085
		ANWC 31914	WA, Australia	Muscle			KJ686079
	caledonicus	None	New Caledonia	Feather	*		KJ686108
		None	New Caledonia	Feather			KJ686106
		None	New Caledonia	Feather			
	indicus	None	Indonesia	Blood			KJ686110
		None	Indonesia	Blood			KJ686104
		None	Indonesia	Blood			KJ686099
		None	Indonesia	Blood			KJ686063
		None	Indonesia	Blood			KJ686075
		None	Indonesia	Blood			KJ686078

	none	Indonesia	Blood			KJ686083
	none	Indonesia	Blood			KJ686080
madagascariensis	TM 61998	South Africa	Muscle		KJ685958	KJ686094
melanopterus	ANWC 8523	Papua New Guinea	Toe pad	KJ685949		
	ANWC 30171	East Timor	Toe pad		KJ685964	KJ686107
melanotus	none	Palmerston North, NZ	Muscle	KJ685951	KJ685963	KJ686113
	none	Palmerston North, NZ	Muscle			KJ686076
	none	Palmerston North, NZ	Muscle			KJ686081
	none	Palmerston North, NZ	Muscle			KJ686101
	none	Palmerston North, NZ	Muscle			KJ686097
	none	Palmerston North, NZ	Muscle			KJ686112
	none	Palmerston North, NZ	Muscle			KJ686096
	none	Northland, NZ	Muscle			KJ686088
	none	Northland, NZ	Muscle			KJ686103
	none	Northland, NZ	Muscle			KJ686093
	none	Northland, NZ	Muscle			KJ686066
	none	Otago, NZ	Muscle			KJ686111
	none	Otago, NZ	Muscle			KJ686077
	none	Otago, NZ	Muscle			KJ686059
	none	Otago, NZ	Muscle			KJ686074
	ANWC 50696	WA, Australia	Muscle			KJ686065
	ANWC 50991	WA, Australia	Muscle			KJ686091
	ANWC 51269	NSW, Australia	Muscle			KJ686086
	ANWC 34145	NSW, Australia	Muscle			KJ686095

	EBU 39915	NSW, Australia	Muscle			KJ686098
	MV 4317	NSW, Australia	Muscle			KJ686061
	MV 4193	Victoria, Australia	Muscle			KJ686060
	MV 4191	Victoria, Australia	Muscle			KJ686062
	MV 4917	Victoria, Australia	Muscle			KJ686102
	MV 5180	Victoria, Australia	Muscle			KJ686105
palliatus	LIV T9048	Sulawesi	Toe pad	*		KJ686090
pelewensis	LIV T9774	Palau	Toe pad	*		KJ686089; KJ68609
poliocephalus	AMNH DOT17002	Captive	Muscle	*	KJ685961	KJ686109
porphyrio	BM 93-0242-T	Spain	Muscle	*	KJ685959	KJ686068
pulverulentus	USNM 578176	Philippines	Toe pad		KJ685962	KJ686087; KJ68610
samoensis	UWBM 90389	American Samoa	Muscle	KJ685954	KJ685957	KJ686070
seistanicus	BMNH 1965.M.2494	Turkey	Toe pad			KJ686072
vitiensis	none	Fiji	Feather			KJ686069
	none	Fiji	Feather			KJ686073

[1] I followed Ripley (1977), Trewick (1996, 1997), and Livezey (1998) for the taxonomic treatment of species and subspecies used i
[2] Specimens of *P. p. melanotus* without museum voucher information were taken from road kill animals or hunting season harves
in Indonesia, *P. p. caledonicus* in New Caledonia and *P. p. vitiensis* in Fiji used blood and/or feathers taken from a wild caught s
capture.
[3] Acronyms for museums are: AMNH (American Museum of Natural History, USA); ANWC (Australian National Wildlife Colle
Spain); BMNH (British Museum of Natural History–Tring Museum, England); EBU (Evolutionary Biology Unit at the Austr
Museum, England); MV (Museum Victoria, Australia); NMNZ (Te Papa Museum, New Zealand); NMW (Natural History M
Museum, South Africa); USNM (Smithsonian Institution, USA); UWBM (Burke Museum of Natural History and Culture, U
Australia).
[4] Specimens of *P. porphyrio* from New Zealand (NZ) and Australia were used for population genetic analyses.

Population differentiation and demographic history

Population level analyses were carried out with two datasets: a) concatenated mitochondrial loci CR and cyt *b*, and b) the autosomal locus BFG–7. Sequence ambiguities at heterozygous sites in BFG–7 that indicated of separate alleles were resolved using PHASE implemented in DnaSP v.5.0 (Librado and Rozas 2009) with the default parameters. To test for intralocus recombination in BFG–7 I used the PHI test (Bruen et al. 2006) implemented in SplitsTree v.4.12.8 (Huson and Bryant 2006). This is a robust test that can reliably detect recombination and report too few false positives (Martin et al. 2011). I calculated the following summary statistics for genetic variation of each population in DnaSP v.5.0 (Librado and Rozas 2009): number of haplotypes (h), nucleotide diversity per site (π), number of segregating sites (S), Watterson's estimator of the per–site population mutation rate (θ_W), Tajima's D statistic (D_T) and Ramos and Rozas' (R_2) test. For each population, D_T was also analysed using 10^3 coalescent simulations conditioned on the sample size and the observed number of segregating sites (Hudson 1990). Demographic expansion was assessed using the R_2 test implemented in DnaSP v.5.0 because it is the most powerful test when dealing with limited sample sizes (Rozas et al. 2003).

Lynch & Crease's pairwise F_{ST} (Lynch and Crease 1990) was calculated in DnaSP v.5.0 using 5000 replicates and a significance level of ≤ 0.05 to test the null hypothesis of panmixia (Raymond and Rousset 1995) between pairs of *Porphyrio porphyrio* populations in Oceania. The level of population genetic structure was tested using an Analysis of Molecular Variance (AMOVA) implemented in Arlequin v.3.5 (Excoffier et al. 2005). To complement the phylogenetic inferences, haplotype networks were constructed using Network v.4.5.1.0 (Bandelt et al. 1999) with median joining to visualize the relationship between haplotypes and their geographical distribution. A Mantel test was conducted for correlation between uncorrected mitochondrial genetic distances estimated in MEGA v.5.2 (Tamura et al. 2011) and linear geographic distances (Jensen et al. 2005) using the ade4 package (Dray and Dufour 2007) implemented in the R programming environment (R Development Core Team 2008) with 10000 permutations.

Results

Phylogenetic analyses

The present study includes wider taxonomic representation than the first molecular phylogenetic study of *Porphyrio* which compared four taxa using a single gene, mitochondrial 12S rRNA (Trewick 1997). *Porphyrio alleni, P. flavirostris* and *P. albus* as well as *P. porphyrio* subspecies: *P. p. porphyrio, P. p. indicus, P. p. poliocephalus* and other Australasian subspecies are now included [note that Trewick (1997) included *P. p. seistanicus* from Turkey]. The complete alignment of six gene fragments contained 4304 bp comprising 816 bp of cyt *b*, 699 bp of CR, 728 bp of 16S, 402 bp of 12S, 868 bp of RAG–1 and 791 bp of BFG–7. No premature stop codons were detected in the two protein–coding genes. Individual gene trees did not reveal spurious sequences or significant conflict among individual phylogenies. Phylogenetic analyses that included just one representative of each species and subspecies that had the most complete gene sets yield similar topologies (results not shown). Topologies from maximum likelihood and Bayesian analyses were congruent for the concatenated dataset. The African species *Porphyrio alleni* is sister to the New World species pair *P. martinica* and *P. flavirostris* (Figure 1C). *Porphyrio porphyrio* did not form a monophyletic group. Instead, it comprised six distinct clades (*porphyrio*, *indicus*, *madagascariensis*, *pulverulentus*, *poliocephalus* [including *seistanicus*], and *melanotus*), and it was paraphyletic with respect to three species-level taxa: *P. mantelli* and *P. hochstetteri* from New Zealand and *P. albus* from Lord Howe Island (Clade A Figure 1C). *Porphyrio p. melanotus* (Clade B Figure 1C) includes the parapatrically distributed subspecies *P. p. bellus, P. p. caledonicus, P. p. samoensis, P. p. vitiensis, P. p. palliatus, P. p. pelewensis, P. p. melanopterus* and *P. p. chathamensis* [the latter was previously demonstrated to be invariant at the 12S locus by Trewick (1997)]. This phylogenetic and spatial structure corresponds with significant differences in colour and size that have been previously described in some detail (Mayr 1949; Ripley 1977; Simmons et al. 1980; Sangster 1998; Taylor 1998). One Indonesian specimen (*P. p. indicus*) did not group with other specimens from this region but instead was more closely related to the clade composed of *P. p. pulverulentus* and *P. albus* (Figure 1C). The close similarity of *P. p. pulverulentus* and *P. albus* sequences indicates a complex history of exchange because their lineage is not recorded in islands between. Sequence data for *P. albus* came from old and rare museum specimens and such ancient DNA

sources have to be treated with caution. However, consistent results were obtained from separate samples and replicate PCRs. There is the possibility of mislabelling of museum specimens, but the white plumage characteristic of *P. albus* is uncommon in other populations.

Molecular dating

Divergence time analysis suggests a mid-Miocene origin of diversification within *Porphyrio* with the split between the lineage leading to the African species *Porphyrio alleni* and other species occurring earliest around 14 (19–9) Mya. Splitting among *Porphyrio porphyrio* "subspecies" was estimated to have occurred about 6 (11–2) Mya with a likely colonization of *P. p. melanotus* in Australasia occurring in the late Pleistocene (600 kya). However, an earlier colonization by a flying *P. porphyrio* at the start of the Pleistocene (~2.5 Mya) resulted in flightless Takahe endemic to New Zealand.

Population genetics structure

The mitochondrial data contained one to four haplotypes (h) in each of the populations sampled (Figure 2A). Nucleotide diversity (π) at sampling localities with $N \geq 2$ was variable, ranging from 0.0 in Northland and Otago, New Zealand, to 0.0059 in Victoria, Australia. Between 0 and 12 segregating sites (S) were observed in each population, yielding a population mutation rate per site (θ_W) from 0.0 to 0.0065 (Table 2). For BFG–7, two alternative haplotypes were identified for alleles possessed by heterozygous individuals. No statistically significant evidence for recombination (p = 0.06) was detected using PHI test. Between 4 and 14 inferred BFG–7 sequences were sampled per population and 1–4 unique haplotypes were found (Table 2). The number of segregating sites in each population varied from 0 to 6. Aside from invariant samples from New South Wales and Western Australia (north), Australia, nucleotide diversity ranged from 0.00069 in Palmerston North, New Zealand, to 0.00440 in Victoria, Australia. This latter population also had the highest inferred population mutation rate per site at 0.00414. No population showed a significantly skewed D_T for mtDNA or nuclear data, although power to reject the null hypothesis of neutrality may have been hampered by small sample sizes. The population size change (R_2) test did not find evidence of demographic expansion of the populations sampled (Table 2).

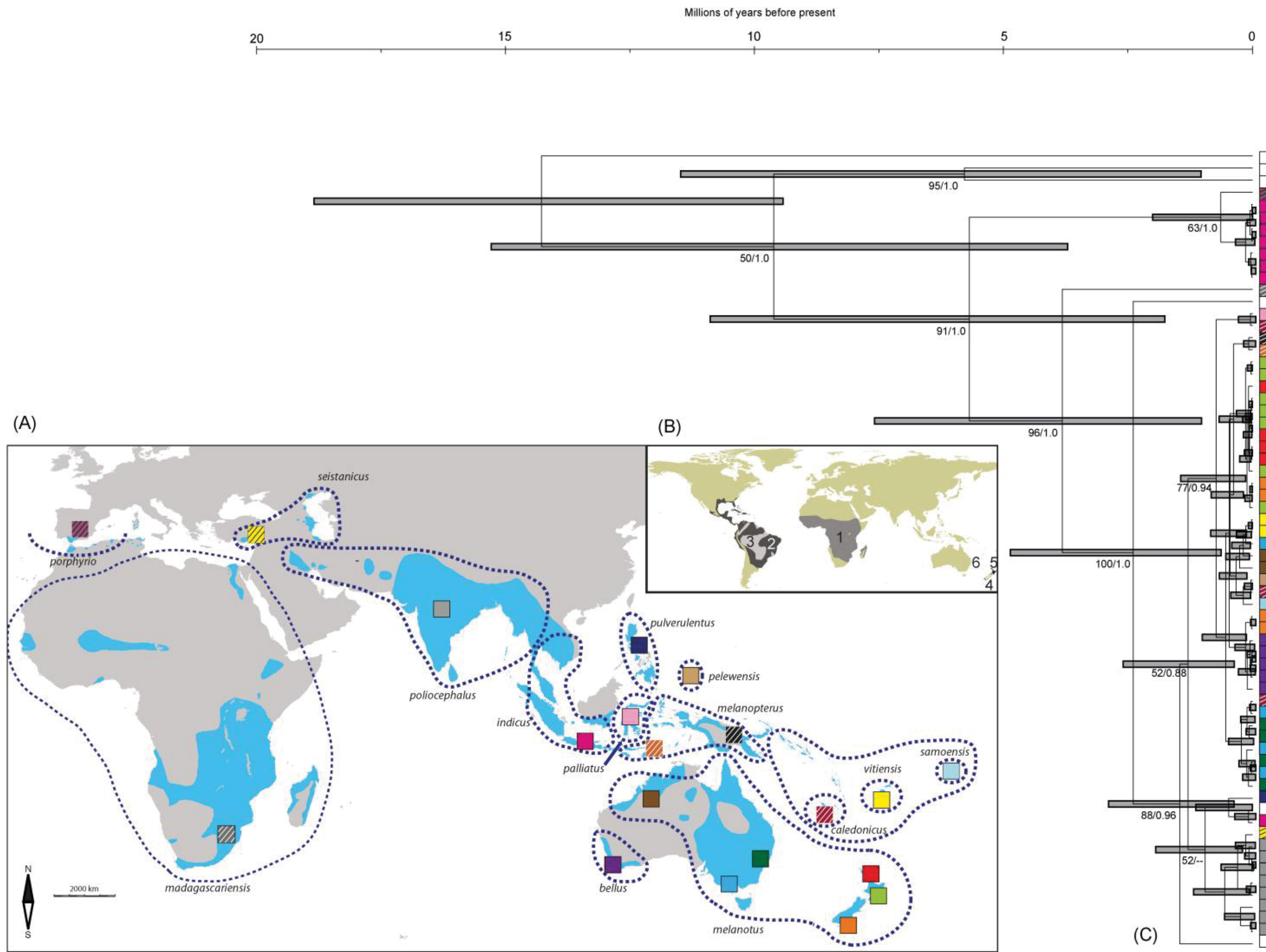

Millions of years before present
20
15
10
5
0
95/1.0
63/1.0
50/1.0
91/1.0
96/1.0
77/0.94
100/1.0
52/0.88
88/0.96
52/--
(A)
(B)
(C)
seistanicus
porphyrio
poliocephalus
pulverulentus
pelewensis
indicus
melanopterus
palliatus
samoensis
vitiensis
caledonicus
madagascariensis
bellus
melanotus
2000 km
N
S

Figure 1. Geographic distribution, phylogenetic relationships and time of divergence of purple swamphens. (**A**) Distributions and sampling localities of the *Porphyrio porphyrio* subspecies included in this study (source: IUCN). Subspecies *caledonicus* and *vitiensis* are included within *samoensis* following Ripley (1997). (**B**) Geographic distributions of the other six *Porphyrio* species around the world. 1 = *P. alleni* (Africa), 2 = *P. martinica* (North and South America), 3 = *P. flavirostris* (South America), 4 = *P. hochstetteri* (South Island, New Zealand), 5 = *P. mantelli* (North Island, New Zealand), 6 = *P. albus* (Lord Howe Island, Australia). (**C**) Chronogram based on analysis of concatenated sequences of mitochondrial and nuclear genes using a relaxed–clock Bayesian analysis in BEAST v.1.7.5. Age constraints were based on estimates by Garcia-R et al. (2014) of the basal divergence of *Porphyrio* and the split between *Porphyrio* and *Amaurornis* in a normal distribution (see methods section). Estimated time of divergence and the 95% HPD intervals of node ages is indicated with a gray bar. Support values for key clades are indicated below the branches and correspond to bootstrap supports (> 50%) and posterior probabilities (> 0.80), respectively.

Panmixia was evident among two localities sampled on the same island and separated by about 500 km: Palmerston North and Northland in North Island, New Zealand. Nevertheless, population genetic structuring was found among other populations more geographically remote from one another, with the exception of some of the comparisons among Western Australia (north) and Victoria, which was probably due to the low sample size (Table 3). Analysis of BFG–7 showed little population genetic structuring (Table 3) among localities at which mtDNA diversity was clearly partitioned. Significantly different values were obtained for most pairwise comparisons among Victoria populations because this location had endemic haplotypes. Consistent with the population pairwise differentiation analysis of the mitochondrial data, most genetic variation was explained by differences among populations (70.5%, $p < 0.0001$). AMOVA of the BFG–7 data indicated that a small but significant (14%, $p < 0.01$) component of variance was attributable among populations. Population geographic structure was evident in the mitochondrial and nuclear haplotype networks (Figure 2B, C), and the Mantel test showed a significant correlation between genetic and geographic distances among populations ($r = 0.508$, $p < 0.05$), even though only about 26% of genetic divergence was explained by geographic distance.

Table 2. Summary of descriptive statistics for mtDNA data (cyt *b* and CR) and the nuclear locus (BFG-7) used in population genet
= number of sequences inferred for nuclear locus, h = number of haplotypes, S = segregating sites, θ_W = population mutation rate p
= Tajima's D statistic, R_2 = Ramos and Rozas statistic. NA = Not Applicable.

Data	Locality	N/2N	h	S	θ_W	π	
mtDNA/BFG–7	Palmerston North	7/14	4/4	3/3	0.00122/0.00119	0.00104/0.00069	-0.65
	Northland	4/8	1/4	0/3	0/0.00146	0/0.00117	NA/-
	Otago	4/8	1/3	0/4	0/0.00195	0/0.00235	NA/(
	Western Australia (south)	5/10	2/3	2/3	0.00096/0.00134	0.00081/0.00076	-0.97
	Western Australia (north)	2/4	2/1	5/0	0.00498/0	0.00498/0	NA/1
	New South Wales	4/6	4/1	7/0	0.00381/0	0.00360/0	-0.38
	Victoria	4/4	3/4	12/6	0.00652/0.00414	0.00592/0.00440	-0.84

Table 3. Pairwise Pairwise comparisons between populations using mtDNA data (above the diagonal) and the nuclear locus BF
represent a program idiosyncrasy due to the small sample size and are effectively zero. *0.01<*p*<0.05; **0.001<*p*<0.01

Locality	Palmerston North	Northland	Otago	Western Australia (south)	Western Australia
Palmerston North		0.083	0.840**	0.883**	0.572*
Northland	-0.030		1.0*	0.945**	0.615
Otago	0.170	0.031		0.950**	0.736
Western Australia (south)	0.018	0.044	0.207*		0.508*
Western Australia (north)	0.038	0.071	0.257	0.0	
New South Wales	0.038	0.071	0.257	0.0	0.0
Victoria	0.170**	0.091*	-0.070	0.163*	0.222*

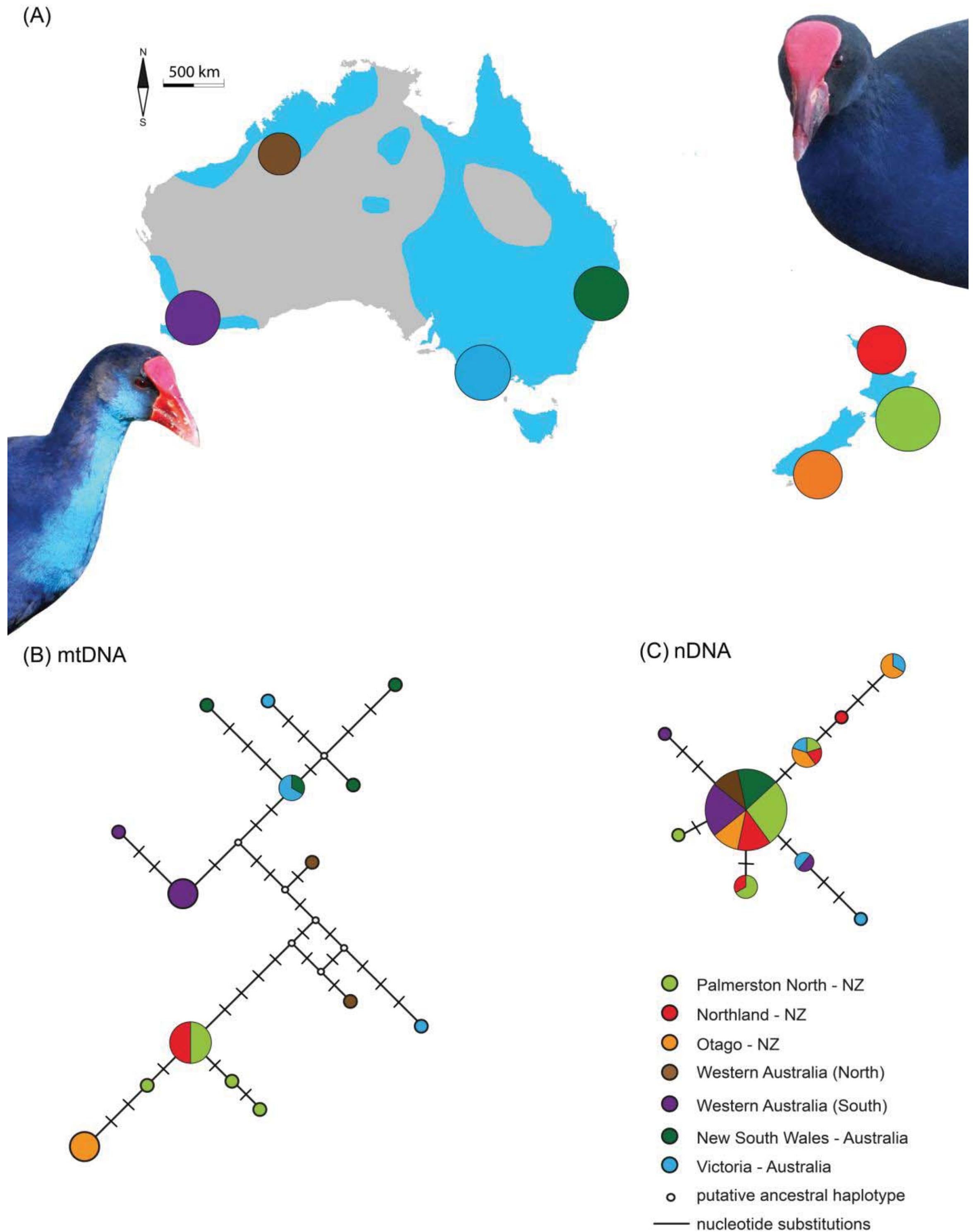


Figure 2. (**A**) Localities in Australia and New Zealand where individuals of *Porphyrio porphyrio* were sampled for population genetic analyses and haplotype networks. Coloured circles are proportional to sample size at each locality. Inset images show plumage colouration of *P. p. bellus* (left) and *P. p. melanotus* (right). Median–joining haplotype networks of (**B**) mitochondrial dataset and (**C**) nuclear gene BFG–7. Circle area is proportional to the number of individuals found for each haplotype. Each line connecting haplotypes indicates a mutational step.

Discussion

Biogeography and evolution of swamphens

The present phylogenetic analyses and molecular dating support independent and temporally non–overlapping colonization events among *Porphyrio* species. This interpretation is, however, based on surviving or recently extinct lineages only; other colonizations are represented by fossils on Oceanic islands (Steadman 1988; Steadman et al. 1999; Steadman 2006) or have left no trace at all. The most likely area of origin of *Porphyrio* is Africa with colonization westward into the Americas and several other colonizations northeastward (Europe, Asia and Oceania) during the Miocene and Pleistocene. The oldest split among the currently recognized *Porphyrio porphyrio* lineage (Clade A Figure 1C) occurred in late Miocene (ca. 6 Mya) giving rise to *P. p. porphyrio* on the Mediterranean coast of Europe and *P. p. indicus* in Indonesia. Further diversification took place during the Pliocene giving rise to *P. p. madagascariensis* in Africa and a radiation into Oceania.

The unique sequence obtained from the extinct *P. albus* of Lord Howe island suggests a close affinity to Philippine *P. p. pulverulentus*, indicating that it was perhaps a white colour variant founded from *P. p. pulverulentus* migrants. The flightless status of *P. albus* appears to be equivocal and the population seems to have been polymorphic for plumage with a high frequency of white individuals (White 1790; Hindwood 1940; Greenway 1967). Aberrations in colour have been found in a number of insular populations, caused perhaps by an allele fixed through a founder effect (Cunningham 1955; Steadman 2006; Uy et al. 2009). White *Porphyrio* occur intermittently, and recent observations include a *P. p. melanotus* individual in Otago, New Zealand (Trewick and Morgan-Richards 2014). The Lord Howe population may have been established from a small number of colonizing individuals from the Philippines during the late Pleistocene (around 500 kya), but this would have involved dispersal from Philippine to Lord Howe over other islands. I remain cautious about the short DNA sequence obtained from *P. albus*.

Despite the appearance that flight is used only infrequently among *P. porphyrio* subspecies, the lineage has dispersed, colonized and established populations multiple

times across open expanses of water. Haplotypes from Indonesia (a specimen from Java is closely related to a specimen from the Philippines) and New Caledonia (specimens from this locality are closely related to specimens from localities as far away as Sulawesi and Palau) support the inference of exchange (Figure 1C). Although rare misplaced haplotypes of this sort might be evidence of ongoing exchange among island populations, they could also be the product of incomplete lineage sorting or past migration events.

Porphyrio p. melanotus (Clade B Figure 1C) appears to have entered Australasia within the last 600 thousand years, but bone deposits show a more recent arrival on some remote islands (Millener 1981; Taylor 1998; Steadman 2006). This includes New Zealand where deposits indicate colonization about 500 years ago after Polynesian settlement (Trewick and Worthy 2001). This dating is much more recent than the estimated divergence of *P. porphyrio* and Takahe lineages (*P. hochstetteri* and *P. mantelli*) that must represent an earlier separate colonization. Within the Australia and New Zealand geographic region, the distribution of genetic variability (phylogenetic analysis, F_{ST}, AMOVA, haplotype networks and Mantel test) indicates the genetic structure of *P. p. melanotus* populations is not homogeneous. This lineage may have originated in Wallacea and eustatic sea level changes could have aided colonization by reducing over–water dispersal distances. Lowered sea level during glacial phases of the Pleistocene reduced the distance between some land areas, including between Papua New Guinea and Australia (Voris 2000; Hall 2009; Jønsson et al. 2010; Wurster et al. 2010; Lohman et al. 2011; Condamine et al. 2013; Irestedt et al. 2013). They did not, however, significantly alter the over water distance between Australia and New Zealand (Graham 2008). This colonization pattern has created the allopatric distribution currently shown in Oceania. I note that higher genetic diversity in Australia than across the Tasman Sea reflects a recent arrival in New Zealand and indicates an influence of distribution due to persistence of habitat rather than geographic distance correlated to human settlement and clearance of forest.

Pairwise differences among Australasian populations were higher in the mtDNA data than for the nuclear DNA gene. This difference may be explained by the higher

mutation rate and lack of recombination in the mitochondrial genome (Neiman and Taylor 2009). However, different population genetic (e.g. background selection), demographic (e.g. effective sex ratio and/or male–biased migration rates), or natural selection also must be considered for those higher mtDNAF_{ST} estimates (Palumbi and Baker 1994; Charlesworth 1998; Stinchcombe and Hoekstra 2007; Charlesworth 2009; Muir et al. 2012). Despite the indication that populations studied have not undergone recent demographic changes, with a marked reduction of genetic variation within populations and increasing genetic differentiation, the swamphens within *P. p. melanotus* of south Western Australia (clade B Figure 1C) show exceptional and not subtle differentiation in plumage colour pattern (Whittell 1934). The current nominate subspecies *P. p. bellus* in south Western Australia has a prominently brighter blue breast and throat colour than *P. p. melanotus*; see images in Figure 2A. Differences in colour, size and other traits are evident among other lineages within the *melanotus* clade (Ripley 1977) and in other clades. For instance, within the *poliocephalus* clade, the Middle Eastern "*seistanicus*" population is grayish compare to individuals from India (see colour photographs in Figure 1C). The mismatch between plumage patterns and the distribution of neutral population genetic markers suggests that differentiation in colour and other traits have arisen rapidly in *Porphyrio* and are subject to selection in local environments (Mayr 1954; Nosil et al. 2009; Feder et al. 2012) or stochastic genetic drift (Clegg et al. 2002a; Clegg et al. 2002b). The lack of sorting at the BFG–7 locus suggests that fixed plumage colour differences among populations are not the result of drift but are perhaps better explained by "purifying" sexual selection.

Selection that results in character divergence among populations can occur without being detectable by neutral genetic markers (Charlesworth and Charlesworth 2009; Nosil et al. 2009). There may be lineages with genomic region(s) involved in adaptive divergence and these regions respond independently to environment pressures via selection (Schneider et al. 1999; Clegg et al. 2002a; Clegg et al. 2002b; Schluter 2009; Via 2009; Cooke et al. 2012). Appearance is a trait that is important in assortative mating by individuals within a population (Schluter 2009; Maan and Seehausen 2011), and is strongly implicated in behaviour of communally breeding *P. p. melanotus* (Jamieson 1988). As such, it may drive monomorphism in local populations. Selection

on mate choice and kin fitness is likely maintaining local population phenotypes in stable frequencies (Andersson et al. 1998; Eaton 2005; Johnsen et al. 2006; Pryke and Griffith 2006; Murphy 2008).

Although *P. p. melanotus* is not the lineage that gave rise to the flightless insular species of New Zealand (*P. mantelli* and *P. hochstetteri*), the recent success of *P. p. melanotus* in reaching several remote islands in the Pacific is testimony to the high success rate of dispersal and colonization. However, the restriction of gene flow evident in F_{ST} values suggests that range expansion is probably episodic, and this enhances the opportunity for speciation and establishment of reproductive barriers. Sexual selection could help drive locus specific evolution without being evident in genes that are with respect to those traits, neutral, and reproductive isolation could evolve as a consequence of local adaptation and selection on characters involved in mate choice and inclusive fitness by mating behaviour.

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Table S1. Taxa, GenBank accession numbers, and original source of data for additional DNA sequences included in thi

Species	Subspecies	Genbank accession number						
		12S	16S	Cyt *b*	CR	BFG–7	RAG–1	
alleni			KC614015	KC614100		KC613893	KC613952	Ga
hochstetteri		NC010092	NC010092	NC010092	NC010092	KC613909	KC613974	M
mantelli		U77144						Tr
martinica			KC614019	KC614103		KC613897	KC613956	Ga
	madagascariensis	U77142						Tr
	melanotus						KC613975	Ga
				HQ916674	HQ896255			Pa
				HQ916670	HQ896247			Pa
				HQ916671	HQ896248			Pa
porphyrio	*poliocephalus*			HQ916672	HQ896249			Pa
				HQ916675	HQ896254			Pa
				HQ916678	HQ896256			Pa
				HQ916676	HQ896252			Pa
	pulverulentus	U77140						Tr
	seistanicus	U77139						Tr

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London Series B-Biological Sciences 352, 1352: 429-46.

Table S2. Primers for PCR and DNA sequencing employed in this study. Primers denoted with * were taken from th
Centre for Molecular Ecology and Evolution, Massey University.

Loci	Primer name	Sequence	Refer
12S	L1873	CCCAACCTAGAGGAGCCTGTTC	Modified from Cooper et
	H2171	GAGGGTGACGGGCGGTATGTACGT	Modified from Cooper et
16S	Av2672F*	GTGGGATGACTTGTTAGT	
	Av3282R*	TGATTATGCTACCTTTGCACGGTCAGGATACC	
	Av3782R*	CGGTCTGAACTCAGATCACGTA	
Cyt *b*	Av15107F*	CATCCGTTGCCCACACATGYCG	
	Av16065R*	GYGRTCTTCYGTCTTTGGTTTACAAGAC	
	L14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	Thomas et al., 1989; Koc
	H15156	AAACTGCAGCCCCTCAGAATGATATTT	Trewick, 1997; Chambers
	TAK2F	CTACTACGGATCATACCTCTAT	This study
	TAK2R	GGTTTGTAATGACTGTAGC	This study
	TAK3R	CCTCCTCATGCTCATTCTAC	This study
	TAK3F	CTTCGTAGGTTATGTCCTACC	This study
CR	Av438F*	TCACGAGAAATCAGCAACCC	
	Av807R*	CTAGKTGTGGGTCAAAGTGCATCAGTG	
	Av1449R*	GAGTRCCCGTGGGGGTGTGGC	
BFG–7	Fib–BI7U	GGAGAAAACAGGACAATGACAATTCAC	Prychitko and Moore, 19
	Fib–BI7L	TCCCCAGTAGTATCTGCCATTAGGGTTT	Prychitko and Moore, 19
	Fib.8R	CCATCCACCACCATCTTCTT	Kimball et al., 2009
RAG–1	R17	CCCTCCTGCTGGTATCCTTGCTT	Groth and Barrowclough,
	R22	GAATGTTCTCAGGATGCCTCCCAT	Groth and Barrowclough,
	R52	CAAGCAGATGAAYTGGAGGC	Irestedt et al., 2001
	R53	TCCATGTCCTTTAAGGCACA	Irestedt et al., 2001

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Appendix. Sequences less than 200 base pairs that cannot be submitted to GenBank. Specimen name is followed by museum voucher when available. Acronyms are the same as found in Table 1.

12S rRNA

>*Porphyrio porphyrio porphyrio*_BM 93-0242-T
AGTACCCGCCTGAGAACTACGAGCACAAACGCTTAAAACTCTAAGGACTTGGCGGT
GCTCCAAACCCACCTAGAGGAGCCTGTTCTGTAATCGATAACCCACGATATACCCA
ACCCCTTCTCGCCCAAAGCAGC

>*Porphyrio porphyrio pelewensis*_LIV T9774
AACTGGGATTAGATACCCCACTATGCTTGGCCCTAAATCCAGATACTCACCACCAC
TAGAGTATCCGCCTGAGAACTACGAGCACAAACGCTTAAAACTCTAAGGACTTGGC
GGTGCCCCAAACCCACCTAGAGGAGCCTGTTCTGTAATCGATAACCCACGATATAC
CCAACCCCTTCTTGCCCAAAGCAGC

>*Porphyrio porphyrio palliatus*_LIV T9048
AACTGGGATTAGATACCCCACTATGCTTGGCCCTAAATCCAGATACTCACTACCAC
TAGAGTATCCGCCTGAGAACTACGAGCACAAACGCTTAAAACTCTAAGGACTTGGC
GGTGCCCCAAACCCACCTAGAGGAGCCTGTTCTGTAATCGATAACCCACGATATAC
CCAACCCCTTCTTGCCCAAAGCAGC

>*Porphyrio porphyrio poliocephalus*_AMNH DOT17002
CGATATACCCAACCCCTTCTTGCCCAAAGCAGCCTACATACCGCCGTCCCCAGCTC
ACCTCCCCTGAGAGCCTAAATAGTGAGCACAACAACACCTCGCTAATAAGACAGGT
CAAGGTATAGCCCATGAAGGGGTAGAAATGGGCTACATTTTCTAAAATAGAAA

>*Porphyrio porphyrio caledonicus*
CGATATACCCAACCCCTTCTTGCCCAAAGCAGCCTACATACCGCCGTCCCCAGCTC
ACCTCCCCTGAGAGCCTAAATAGTGAGCACAACAACACCTCGCTAATAAGACAGGT
CAAGGTATAGCCCATGAAGGGGTAGAAATGGGCTACATTTTCTAAAATAGAAA

Cyt *b*

>*Porphyrio mantelli*_NMNZ DM7930
GGATCATACCTCTATAAAGAAACCTGAAACACAGGAATCATCCTACTACTCACCCT
AATAGCCACTGCCTTCGTAGGCTATGTCCTACCATGAGGACAAATATCCTTCTGAG
GCGCTACAGTCATTACAAACCTATTCTCAGCCATC

The role of gene flow in speciation from a colonizing rail bird in Oceania[1]

[1] In preparation: Garcia-R JC, Joseph L, Lux F, Adcock G, Reid J, Trewick SA.

Abstract

Colonisation often involves marked founder effects associated with population bottlenecks that reduce within–population genetic diversity and increase genetic differentiation among populations. Studies of fragmented areas, such as islands in archipelagos, provided important insights of the microevolutionary processes that drive the early stages of diversification. I examined the role of gene flow in the genetic variation of the banded rail (*Gallirallus philippensis*) in Oceania to understand the factors that promote speciation within this bird lineage. The results show that the banded rail has a high genetic variability in its ancestral area or oldest native range, the Philippines archipelago, and the geographically close archipelagos Palau and Wallacea, while the geographically distant and large island/continent of Australia showed somewhat lower but still considerable genetic variability. Other archipelagos sampled were found to have less genetic diversity and haplotypes closely related to Wallacea (Bismarck, Samoa, Vanuatu, New Caledonia, New Zealand, Cocos) and Australia (New Zealand, Samoa, Fiji, Cocos). I found that nucleotide diversity declined with respect to the colonisation of new archipelagos but not the haplotype diversity, which remained roughly the same. However, both nucleotide and haplotype diversities were positively correlated with archipelago area. These results are mostly concordant with abrupt genetic changes due to founder events with the Philippines as the most divergent region and multiple colonisation events into Australia from Wallacea and Bismarck. Likewise, the data suggests that this large island/continent worked as a refugium during the Last Glacial Maximum and an immediate source of birds for other islands in the Pacific.

Keywords: archipelagos, Oceania, colonisation, gene flow, islands, subspecies

Introduction

Dispersal is an important feature of biological evolution and diversification (Roy and Goldberg 2007). The movement of individuals may be largely stochastic (but see Edelaar et al. 2008; Edelaar and Bolnick 2012) whereas success in colonisation and establishment may be strongly influenced by availability of niche space with few competitors (i.e. colonisation opportunity) and hence many new ecological opportunities (i.e. a wide resource spectrum with many adaptive peaks allowing more efficient use of resources) (Trewick and Gibb 2010). Colonisation of novel habitats plays an important role in many ecological processes, from ecological succession to population dynamics and range expansion. However, the capacity for successful dispersion, colonisation and establishment of populations in new habitats can lead to some population genetic changes (Clegg et al. 2002).

Colonisation often involves perceptible founder effects and genetic bottlenecks with abrupt or gradual reduction of genetic variation and increase of genetic differentiation that can influence speciation (Schneider 2000). Founder populations are generally associated with less genetic diversity because they contain a small fraction of the genotypic variation. Founder populations are more susceptible to inbreeding and in some circumstances are subjected to reduced predation and competition pressure (Frankham 1997; Dlugosch and Parker 2008). Nonetheless, some studies have found that populations of species colonizing novel habitats harbour genetic diversity as high as or higher than that found in any single source population due to mixture of individuals from geographically and genetically distinct origins, gene flow from multiple independent dispersions or recombination between divergent genomes (Young et al. 1996; Grant et al. 2001; Kolbe et al. 2004).

Founder events are highly likely in habitat patches such as oceanic islands and genetic change can be estimated by comparisons of the level of genetic variation between conspecific populations despite the lack of information about timing and sequence of colonisations. Successful colonisation and establishment on islands is influenced by the life histories and reproductive systems of the vagrant species. However, theory predicts that populations from mainland or large islands are more likely to be a source of migrants rather than sink areas and colonisation rates are related to the distance from the sources. As a result, small and peripheral island populations are associated with genetic

consequences of colonisation and may contain less genetic variation (Mayr 1954; MacArthur and Wilson 1967; Diamond et al. 1976; Provine 1989; Hubbell 2001).

The bird genus *Gallirallus* comprises widespread volant and local endemic flightless species on islands in the western Pacific. Diversification of this lineage appears to involve colonisation of oceanic islands and subsequent morphological differentiation sometimes leading evolution of flightless species that look different from their flying relatives but similar to one other flightless species (Trewick 2011). Previous studies of *Gallirallus* using molecular data have shown relatively low levels of interspecific genetic divergence within the genus (Kirchman 2009; Kirchman 2012) with each flightless species inferred as having evolved from a flying colonist ancestor (i.e. following a sequence of colonisation events) rather than from another flightless rail (Beauchamp 1989; Trewick 1997; Mayr and Diamond 2001). This indicates interplay between the frequency with which individual birds move between island populations (gene flow) and the intensity of selection acting within island populations.

The widespread flighted banded rail (*Gallirallus philippensis*) has a broad range of habitat tolerances and over 20 subspecies based mainly on plumage differences have been acknowledged (Ripley 1977; Schodde and Naurois 1982; Elliott 1987; Taylor 1998). The graded level of morphological differentiation suggests spatial genetic structure caused by restriction of gene flow among some populations. Although the broad distribution of the banded rail over large bodies of water suggest a great propensity for dispersal and colonisation on isolated islands, their migratory habits are not clear. This organism provides an excellent model to contribute to the integration of ecological and historical approaches using fine–scale phylogeographical analyses. Understanding how the spatial and temporal aspects of microevolution operate in the banded rail is key to recognising how *Gallirallus* speciated in islands of the south–west Pacific.

Traditionally, the history of colonisation events has been inferred from genetic data through a combination of population genetic summary statistics that describe the quantity of diversity (allelic diversity), population subdivision (F_{ST}) or spatial arrangement of genetic variation (AMOVA). More recently, coalescent models of gene flow have provided the opportunity to infer demographic history from population

genetic data. These model–based approaches represent a potentially powerful alternative to traditional population genetic analyses for studying gene flow and colonisation that help our understanding of evolutionary processes that occur during population divergence or speciation. Compared to simple descriptive measures of population differentiation, this approach incorporates genetic stochasticity by sampling from the range of genealogies that are consistent with the data (Hey and Nielsen 2004).

I examined the patterns of genetic variation among populations to make inferences about the colonisation process of banded rail in Oceania. I applied both traditional population genetic analyses and coalescent models to obtain an overall view of wide–ranging genetic diversity of banded rail that allows reconstruction of migration and colonisation history. My goal was to examine genetic variation in populations all around the geographic range of banded rail in Oceania and the Philippines. If founder events are important in this system I expected these populations to present bottleneck signals. The aims of this study were to: (i) quantify genetic differentiation within and between populations of banded rail in Oceania and the Philippines, (ii) determine the genetic structure in its geographical distribution, and (iii) discern pathways of colonisation and patterns of gene flow among geographically structured groupings.

Materials and Methods

Laboratory techniques

Genomic DNA was extracted from feathers, faeces, toe pads, blood and fresh muscle tissue (Table S1). Ancient DNA (aDNA) extraction from toe pads was carried out in a dedicated ancient DNA laboratory at Ecology Group, Massey University (http://evolves.massey.ac.nz/DNA%20Toolkit.htm) using either standard phenol–chloroform or the Qiagen QiAMP DNA Minikit (Qiagen). DNA from blood, muscle and feathers were obtained using the Qiagen QIAamp tissue kit (Qiagen) and DNA from faeces was extracted using either phenol–chloroform method or the Nucleospin soil kit (Macherey–Nagel).

Genetic data and analyses

Published Control Region (CR) DNA sequences for banded rail from Vanuatu and other Pacific islands (Kirchman and Franklin 2007; Kirchman 2009) was augmented with additional data that I collected following amplification procedures and primers

previously published (Kirchman 2009; Ozaki et al. 2010). This study includes a wide sampling of localities (48), subspecies (15) and archipelagos (12) (Figure 1, Table 1). I excluded three sequences from the published dataset (EF219120, EF219117 and EF219129) because they are different from haplotypes 2 (EF219120) and 27 (EF219117), as described by Kirchman (2009), and there is no detailed information about the source of the sample EF219129. Additional samples were obtained from multiple localities in New Caledonia, North Island (New Zealand), Samoa, Australia and several islands in Fiji. New and previously unsampled localities corresponding to Cocos island, Niue island, South Island (New Zealand) and Sumba (Indonesia) ensure a graded spatial intra– and inter–archipelagic variation, which provided an ideal array of spatial heterogeneity to explore gene flow at different geographic scales (Table 1). Control Region sequences are available under GenBank Accession numbers in Table S1. I calculated the following summary statistics for genetic variation of each population, archipelago and subspecies in DnaSP v.5.0 (Librado and Rozas 2009): number of haplotypes (h), number of segregating sites (S), haplotype diversity (Hd), nucleotide diversity per site (π) and Watterson's estimator of the per–site population mutation rate (θ_W). Inferences of population expansion were made using Tajima's D (Tajima 1989b) statistic (D_T), Fu's (*Fs*) test (Fu 1997) and R_2 test (Ramos-Onsins and Rozas 2002; Ramirez-Soriano et al. 2008). Tajima's D is based on the differences between the number of segregating sites and the average number of nucleotide differences and *Fs* statistic of Fu is based on the probability of having a number of haplotypes greater or equal to the observed number of samples drawn from a constant–sized population. To complement these methods, I calculated the R_2 statistic, which is based on the difference between the number of singleton mutations and the average number of nucleotide differences. The significance of R_2 was obtained by examining the null distribution of 5000 coalescent simulations in DnaSP. Significantly negative values of statistical tests of neutrality (D_T and Fu's *Fs*) reveal an excess of rare nucleotides segregating in the sample which could be caused by a past demographic expansion event (Tajima 1989a), initial recovery of variation after a selective sweep (Maynard Smith and Haigh 1974; Braverman et al. 1995) or bottleneck (Fay and Wu 1999; Hammer et al. 2004), or low incidence of gene flow from other populations. Significantly positive values are due to an excess of common variants relative to rare ones which may be caused by balancing selection or recent decrease in population size

(bottleneck). Significantly large negative D_T and/or Fu's *Fs* values and significantly positive R_2 values are taken as evidence of population expansion.

Genetic divergence between each pairwise comparison was quantified using Lynch & Crease's F_{ST} (Lynch and Crease 1990) in DnaSP v.5.0 with 5000 replicates and a significance level of ≤ 0.05. Haplotype networks were constructed in PopART v. 1.0 using median joining to visualize the relationship between haplotypes and their geographical distribution, while a Mantel test was conducted for correlation between genetic (Wright's F_{ST}) and geographical distances (km) using the ade4 package (Dray and Dufour 2007) implemented from the R programming environment with 10000 permutations. Correlation coefficients were also calculated to estimate the relationship between nucleotide diversity per site (π) and haplotype diversity (Hd) among area of archipelagos (km^2). Archipelago areas, haplotype diversity and nucleotide diversity per site were log_{10} transformed prior to the analyses.

I obtained estimates of the effective population sizes (*Ne*), asymmetric pairwise migration rates (*Nm*) and time since population divergence (*t*) using coalescence analyses under an isolation–with–migration model implemented in the program IMa (Hey and Nielsen 2007). To convert parameter estimates of IMa into demographic units for the CR data, an inheritance scalar of 0.25 and a mean generation time of two years were assumed according to estimates for banded rail (Dunlop 1970; Garnett et al. 2011). I used the Hasegawa, Kishino and Yano (HKY) model of substitution and several preliminary MCMC simulations with broad priors were performed to set appropriate bounds for each parameter. I compared the convergence of the marginal distributions of each parameter among multiple runs and obtained the credibility intervals based on 90% highest posterior density (HPD).

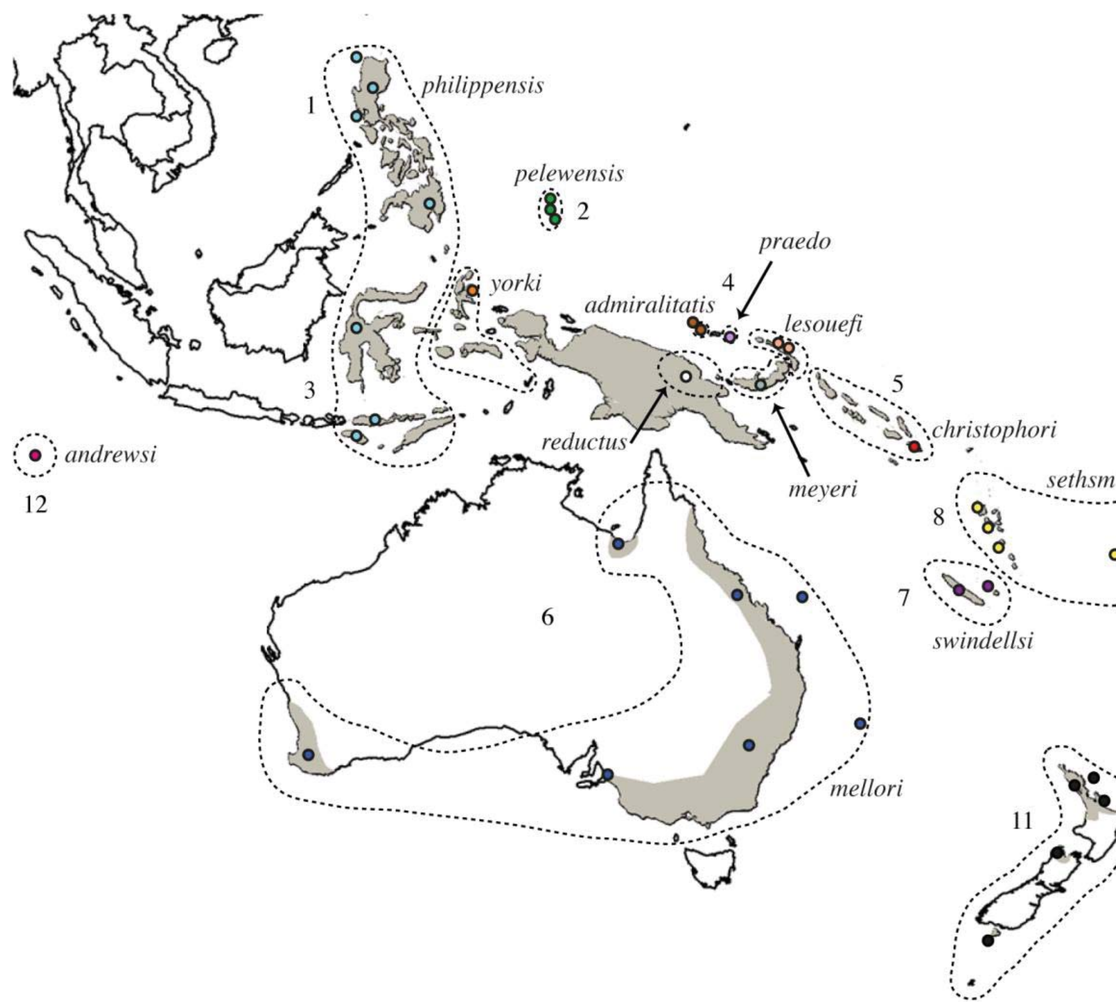

Figure 1. Map of the Australasia region showing sampling localities (small coloured circles) and sampled banded rail (*G. ph*
archipelagos where: 1 = Philippines, 2 = Palau, 3 = Wallacea, 4 = Bismarck, 5 = Solomon, 6 = Australia, 7 = New Caledonia, 8 =
Zealand, 12 = Cocos). Gray shade indicates geographical distribution of the species.

Table 1. Archipelago, subspecies, localities, sample size, haplotype numbers, and original source of the Control Regior

Archipelago	subspecies	localities	N	Haplotypes	Source
Cocos	*andrewsi*	Cocos Island	30	24, 39, 42	This study
Philippines	*philippensis*	Batan	1	30	Kirchman 2009
		Luzon	2	31, 33	Kirchman 2009
		Lagangilang	1	32	Kirchman 2009
		Mindanao	6	35, 36, 37, 38	Kirchman 2009
Wallacea		Sulawesi	3	1, 4, 11	Kirchman 2009
		Flores	1	1	Kirchman 2009
		Sumba	3	20, 24, 49	This study
	yorki	Halmahera	1	23	Kirchman 2009
Palau	*pelewensis*	Garakayo	1	28	Kirchman 2009
		Koror	1	34	Kirchman 2009
		Peleliu	1	28	Kirchman 2009
Bismarck	*reductus*	New Guinea	2	3, 10	Kirchman 2009
	admiralitatis	Pityilu	1	29	Kirchman 2009
		Ninigo	1	29	Kirchman 2009
	praedo	Skoki	1	12	Kirchman 2009
	lesouefi	Wanton	1	13	Kirchman 2009
		Boang	1	1	Kirchman 2009
	meyeri	New Britain	2	1	Kirchman 2009
Solomon	*christophori*	San Christobal	1	16	Kirchman 2009
Australia	*mellori*	NSW	9	14, 15, 22, 24, 26, 40, 45, 46	Kirchman and Fra
		Lord Howe island	1	41	This study
		SA	1	24	This study
		QLD	4	15, 22, 26	Kirchman 2009; T

		Heron island	2	22	Kirchman 2009
		NT	2	21, 25	Kirchman 2009
		WA	3	20, 24	This study
		?	3	1, 15, 24	Kirchman 2009
New Zealand	*assimilis*	Northland	5	15, 40, 44	This study
		Great Barrier island	5	26, 40	This study
		Whakatane	1	40	This study
		Golden Bay	12	40, 41, 48	This study
		Kundy island	1	40	This study
New Caledonia	*swindellsi*	New Caledonia	2	1	Kirchman 2009; T
		Loyalty	1	2	Kirchman 2009
Fiji		Taveuni	4	2, 43	Kirchman 2009; T
		Vorovoro	2	9, 40	This study
		Vatulele	2	9, 15	This study
	sethsmithi	Ono	2	9, 15	This study
Vanuatu		Santo	10	2, 7, 8, 17, 18, 27	Kirchman and Fra
		Efate	5	2, 6	Kirchman and Fra
		Uripiv	4	2, 9, 19	Kirchman and Fra
Samoa	*goodsoni*	Upolu	1	1	This study
		Nofoa	1	40	This study
		Tutuila	3	20, 47	Kirchman 2009; T
		Manua	1	20	Kirchman 2009
		Ofu	1	20	Kirchman 2009
		Niue	3	1	This study

To obtain *Ne* from θ, a mutation rate, μ, of 4.7×10^{-8} per site per year was applied for this CR data. The variation in mutation rate among mitochondrial genes has not been studied in these birds, so to obtain this approximation, I assumed the same mutation and substitution rate for CR (Ho et al. 2005). Although, substitution rate varies dramatically among mitochondrial genes in animals and variation in rates of mutation is less dramatic, some analyses suggested that genes near origins of replication may have elevated mutation rates due to the slightly longer exposure of single–stranded DNA to free oxygen radicals in the matrix of this organelle (Brown et al. 1979; Moritz et al. 1987; Ingman et al. 2000; Broughton and Reneau 2006). I compared percent divergence in CR with percent divergence in the cytochrome *b* (cyt *b*) coding region sequences for two pairwise comparisons of banded rail populations in Australia. Weir & Schluter (2008) calibrated a mutation rate of 2.1% sequence divergence per My for cyt *b* mtDNA coding region across several avian orders. Based on the pairwise comparisons of intraspecific diversity of *G. philippensis*, CR mutates at a rate (0.9% ± 0.3) that is about 4.5 times faster than cyt *b* (0.2% ± 0.1) coding region (range = 4.0 to 6.0 times faster). Thus, I estimated that the CR evolves at a rate of 9.5 % sequence divergence per My or at a rate of 4.7×10^{-8} substitutions per site per year (3.0×10^{-8} to 12×10^{-8} s/s/y).

Results

Genetic diversity

I analysed 433 bp of CR sequence data for 152 individuals of *G. philippensis* across Oceania and the Philippines. I identified 49 haplotypes of which 38 were previously reported by Kirchman (2009). Three haplotypes were widely distributed in the archipelagos Wallacea, Bismarck, New Zealand, New Caledonia and Samoa (Hap 1); Cocos, Wallacea, Australia and New Zealand (Hap 24); and Australia, New Zealand, Fiji and Samoa (Hap 40), while 32 endemic haplotypes were found in Philippines (Hap 30, 31, 32, 33, 35, 37, 38), Australia (Hap 14, 21, 25, 45, 46), Vanuatu (Hap 6, 7, 8, 17, 18, 19), Bismarck (Hap 3, 10, 12, 13), Wallacea (Hap 4, 11, 23, 49), New Zealand (Hap 44, 48), Solomon (Hap 16), Palau (Hap 34), Fiji (Hap 43), and Samoa (Hap 47).

Nucleotide diversity (π) in sampling localities with $N \geq 2$ ranged from 0.0 (Niue, New Britain, New Caledonia, Heron Island) to 0.0153 (New Guinea). At archipelago and subspecies level, the Cocos island (π = 0.00249) and *G. p. admiralitatis* and *G. p. meyeri* subspecies (π = 0.0) showed the lowest values contrasting with Palau and *G. p.*

philippensis (0.0246 and 0.0256, respectively; Table 2). Between 0 and 9 segregating sites (S) were observed in each population, yielding a population mutation rate per site (θ_W) from 0.0 to 0.0153. The Philippines, Wallacea, Vanuatu and Palau and their respective subspecies presented the highest number of segreating sites (S = 12 for all archipelagos; and S = 18, 13 and 12 for *G. p. philippensis, G. p. sethsmithi* and *G. p. pelewensis*, respectively; Table 2). Nucleotide diversity declined with respect to the colonisation of isolated archipelagos but haplotype diversity remained approximately the same (Figures 2a, b). None of the populations and archipelagos showed any significant skew in values of D_T, with the exception of Fu's *Fs* test in the NSW deme and Australia, New Zealand and Vanuatu archipelagos. These Fu's results indicate departures from the Wright–Fisher neutral model for these populations. Subspecies showing significant negative deviation from neutality according to the Fu's *Fs* test were *G. p. philippensis, G. p. mellori, G. p. assimilis* and *G. p. sethsmithi*. Two populations and one archipelago had statistically significant R_2 values: NSW ($R_2 = 0.137$, $p < 0.001$) and Santo ($R_2 = 0.112$, $p < 0.001$) at population level and Bismarck ($R_2 = 0.116$, $p < 0.05$) at archipelago level (Table 2).

Genetic structure and coalescent analyses

I ran a preliminary analysis to measure pairwise F_{ST} among localities within a given archipelago. There were no significant departures of pairwise F_{ST} values from 0, with the exception of comparisons among localities in New Zealand and Philippines (results not shown). These F_{ST} results may reflect low sampling size in some cases. As a result I grouped samples from each dataset into putative populations by putting individuals from the same archipelago or the same subspecies together in order to perform subsequent analyses. Genetic differentiation between pairs of archipelagos and subspecies showed that New Caledonia and its corresponding subspecies (*G. p. swindellsi*) provide no evidence of population genetic structuring when compare with Vanuatu, Fiji, Samoa, Australia, Palau and Wallacea and the subspecies in those archipelagos (*G. p. sethsmithi, G. p. goodsoni, G. p. mellori, G. p. pelewensis* and *G. p. philippensis*; Table 3), but this is probably due to the low sample size (N = 3) in New Caledonia. On the other hand, the smallest values of highly significant divergence ($p < 0.001$) in terms of F_{ST}, were mostly found in comparisons among the Australia archipelago, as well as among the New Zealand archipelago, while the biggest values of highly significant divergence ($p < 0.001$) were mostly found among Philippines

archipelago. Estimates of divergences at subspecies level showed the smallest values of highly significant divergence ($p < 0.001$) among comparisons of *G. p. mellori* and the biggest values of differentiation ($p < 0.001$) mostly among comparisons of *G. p. andrewsi*.

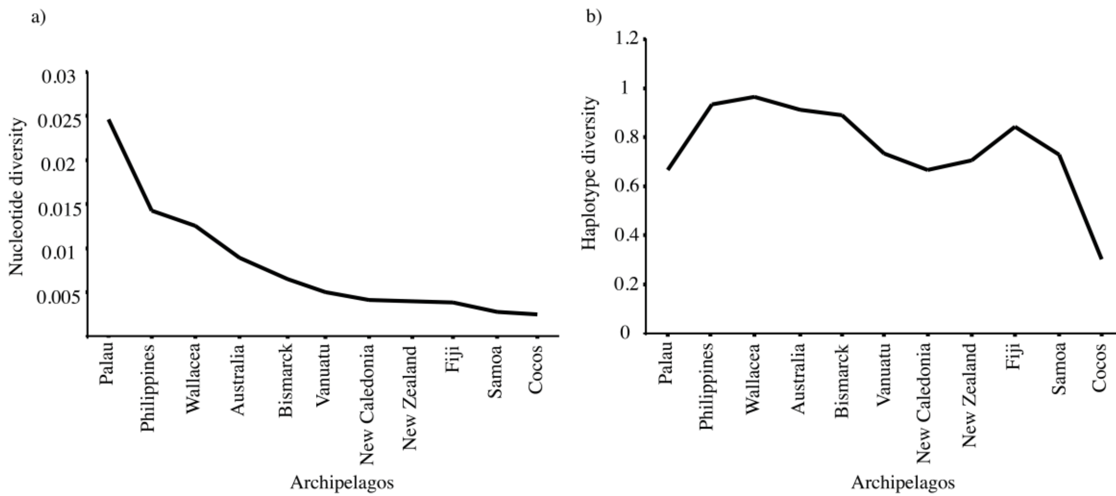

Figure 2. Genetic diversity plots of banded rail measured by (a) nucleotide diversity and (b) haplotype diversity among archipelagos.

Geographical association of haplotypes visualized with a network identified a similar structure with haplotypes from the Philippines archipelago being the most divergent from all others and haplotypes found in Australia and New Zealand occurring throughout the haplotype network (Figure 3). There was a positive correlation between the genetic difference (F_{ST}) and the spatial scale at archipelago level supported by the Mantel test (r = 0.382, $p = 0.012$; Figure 4). Furthermore, I tested for a relationship between nucleotide diversity per site (π) and archipelago area (km^2), and found a positive correlation (r = 0.75, $p < 0.01$) but only when the Palau archipelago was excluded from the analysis. There was also a positive correlation between haplotype diversity (Hd) and archipelago area (km^2), without excluding Palau archipelago (r = 0.83, $p < 0.01$).

Table 2. Genetic polymorphism estimates for three datasets analysed: 1) individual localities with more than tw archipelago, and 3) subspecies with more than two samples. N = sample sizes, h = haplotype numbers, S = segregatir population mutation rate per site, π = nucleotide diversity per site, D_T = Tajima's D test, Fs = Fu's *F*s test, R_2 = Applicable. * = 0.01< P < 0.05; ** = 0.001 < P < 0.01; *** = < 0.001.

Dataset partitions	**N**	**h**	**S**	**Hd**	θ_W	**π**	D_T
ARCHIPELAGOS/LOCALITIES							
Cocos	30	3	4	0.301	0.0025	0.00249	-0.048
Cocos Island	30	3	4	0.301	0.0025	0.00249	-0.048
Philippines	10	8	12	0.933	0.013	0.0143	0.428
Mindanao	6	4	3	0.8	0.004	0.0045	0.6
Luzon	2	2	2	1	0.00615	0.00615	NA
Wallacea	8	7	12	0.964	0.0159	0.0126	-1.032
Sulawesi	3	3	2	1	0.0041	0.0041	NA
Sumba	3	3	3	1	0.005	0.005	NA
Australia	22	11	10	0.913	0.0094	0.009	-0.165
NSW	9	8	9	0.972	0.0101	0.0097	-0.201
QLD	4	3	5	0.833	0.0083	0.0087	0.371
WA	3	2	1	0.667	0.0016	0.0016	NA
Heron island	2	1	0	0	0	0	NA
NT	2	2	3	1	0.0092	0.0092	NA
New Zealand	27	8	7	0.707	0.0055	0.004	-0.853
Northland	5	3	2	0.7	0.0022	0.0018	-0.972
Great Barrier island	5	2	2	0.4	0.0022	0.0018	-0.972
Golden Bay	12	3	2	0.345	0.0015	0.0008	-1.429

Fiji	10	5	4	0.844	0.0032	0.0039	0.806
Taveuni	4	2	1	0.5	0.0012	0.0011	-0.612
Vorovoro	2	2	2	1	0.0046	0.0046	NA
Vatulele	2	2	2	1	0.0046	0.0046	NA
Ono	2	2	2	1	0.0046	0.0046	NA
Vanuatu	19	9	12	0.731	0.0079	0.0051	-1.271
Santo	10	5	4	0.667	0.0032	0.0022	-1.244
Efate	5	4	5	0.9	0.0055	0.005	-0.561
Uripiv	4	2	6	0.667	0.0075	0.0092	2.156
Samoa	10	4	2	0.733	0.0022	0.0028	0.931
Tutuila	3	2	1	0.667	0.002	0.002	NA
Niue	3	1	0	0	0	0	NA
Bismarck	9	6	8	0.889	0.00906	0.00684	-1.112
New Guinea	2	2	5	1	0.0153	0.0153	NA
New Britain	2	1	0	0	0	0	NA
Palau	3	2	12	0.667	0.0246	0.0246	NA
New Caledonia	3	2	2	0.667	0.0041	0.0041	NA
New Caledonia	2	1	0	0	0	0	NA
SUBSPECIES							
Andrewsi	30	3	4	0.301	0.0025	0.00249	-0.048
Philippensis	17	14	18	0.971	0.0183	0.0256	1.548
Pelewensis	3	2	12	0.667	0.0246	0.0246	NA
Reductus	2	2	5	1	0.0153	0.0153	NA
Admiralitatis	2	1	0	0	0	0	NA

Lesouefi	2	2	1	1	0.0031	0.0031	NA
Meyeri	2	1	0	0	0	0	NA
Mellori	22	11	10	0.913	0.0094	0.009	-0.165
Assimilis	27	8	7	0.707	0.0055	0.004	-0.853
Swindellsi	3	2	2	0.667	0.0041	0.0041	NA
Sethsmithi	29	12	13	0.788	0.0076	0.0049	-1.207
Goodsoni	10	4	2	0.733	0.0022	0.0028	-0.931

Table 3. Pairwise F_{ST} values between 11 (Solomon excluded) major geographical regions (above the diagonal) and
diagonal and bold names and values). * = 0.01< P < 0.05; ** = 0.001 < P < 0.01; *** = < 0.001.

Subspecies		***A***	***B***	***C***	***D***	***E***	***F***		***G***	***H***
	Archipelagos	1				2	3	4	5	6
reductus (A)										
admiralitatis (B)	Bismarck (1)	**0.285**				0	0.328***	0.230**	0.184*	0.043
lesouefi (C)		**0**	**0.8**							
meyeri (D)		**0**	**1**	**0**						
swindellsi (E)	New Caledonia (2)	**-0.05**	**0.714**	**0**	**0**		0.131	0.028	0.178	0.029
sethsmithi (F)	Vanuatu (3)	**0.137***	**0.66***	**0.425**	**0.522**	**0.087**		0.055	0.476***	0.284***
	Fiji (4)								0.424***	0.246***
goodsoni (G)	Samoa (5)	**0.076***	**0.831***	**0.203**	**0.349**	**0.178**	**0.452*****			0.012
philippensis (H)	Wallacea (6) Philippines (7)	**0.243***	**0.387**	**0.4**	**0.431**	**0.37**	**0.384*****		**0.39****	
pelewensis (I)	Palau (8)	**0.29**	**0.368**	**0.4**	**0.428**	**0.382**	**0.383*****		**0.328****	**0.049***
assimilis (J)	New Zealand (9)	**0.185****	**0.771*****	**0.449**	**0.59**	**0.399***	**0.464*****		**0.362*****	**0.339*****
mellori (K)	Australia (10)	**0.09****	**0.544**	**0.236**	**0.299**	**0.206**	**0.352*****		**0.082***	**0.299*****
andrewsi (L)	Cocos (11)	**0.274****	**0.928*****	**0.591****	**0.831***	**0.539****	**0.686*****		**0.289****	**0.448*****

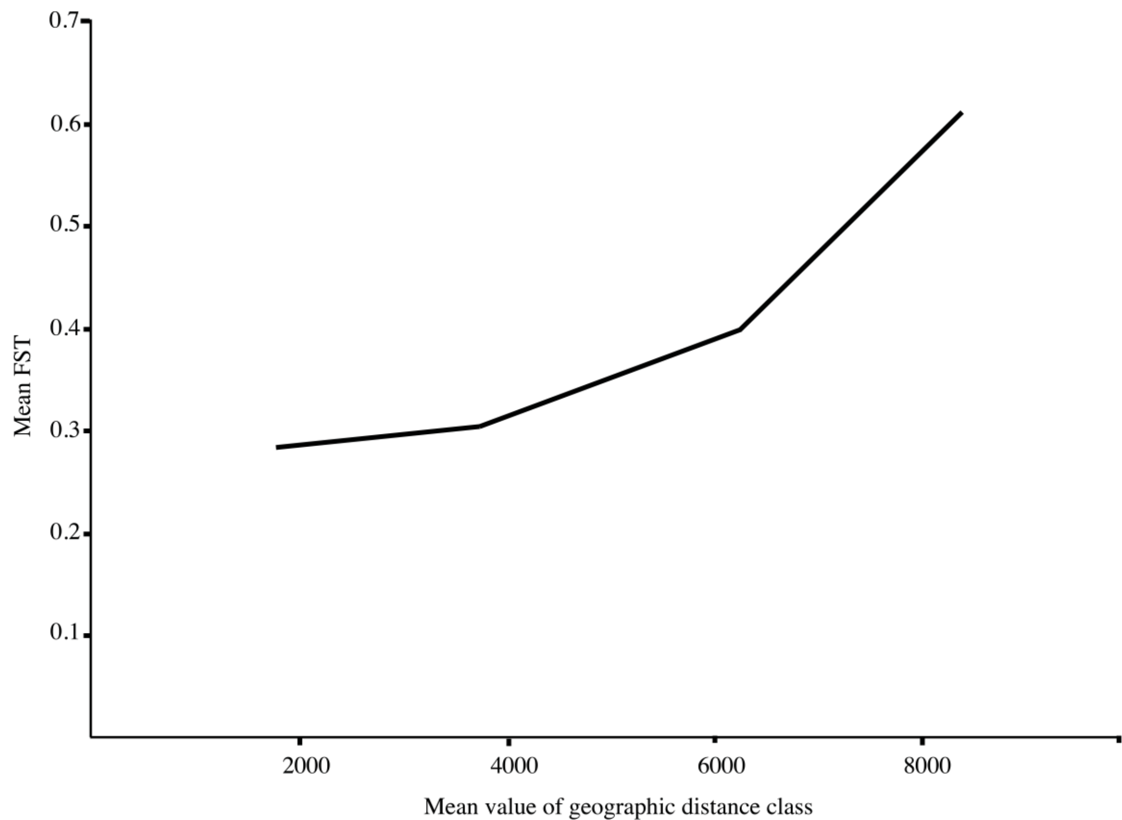

Figure 3. Distogram showing the relationship between mean pairwise F_{ST} within distance classes.

Results from the coalescent model showed asymmetric gene flow (*Nm*) among populations grouped into archipelagos with the lowest estimated migration rates ($Nm < 0.01$) being those towards the most isolated archipelagos, Samoa and Cocos, from their nearest adjacent archipelagos, Fiji and Wallacea, respectively. The largest migration rates ($Nm > 1$) were indicated as being into Vanuatu from Australia and New Zealand (Figure 5, Table S2). Overall, based on these results, I suggest that banded rails have followed a stepping–stone pattern with Australia/New Zealand acting as source of dispersal for other remote islands in Oceania. The results suggest spatially structured colonisation, from the biggest islands to the nearest island groups, and onwards to more remote islands, with only one case of back–gene flow to Australia and New Zealand from Vanuatu.

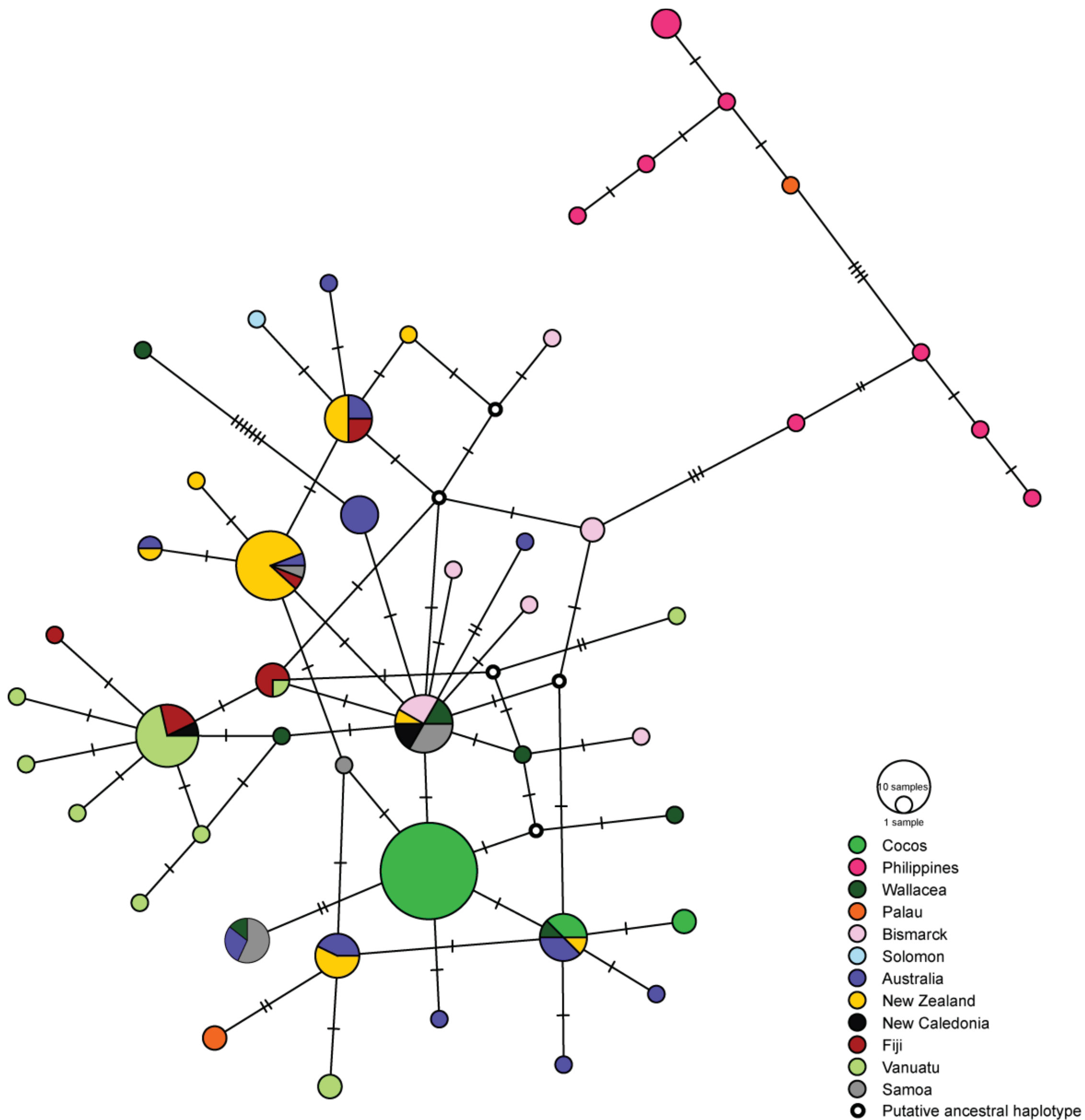

Figure 4. Median–joining haplotype network. The size of the circle is proportional to the frequency of individuals found for each haplotype. Numbers of substitutions are indicated on each line connecting the haplotypes. Colours correspond to 12 major geographical regions representing archipelagos dataset.

Discussion

The results show that colonisation events in Oceania for the banded rail have had a limited effect on levels of divergence. Haplotype diversity within archipelagos did not show significant reductions after colonisation events. This measure is an indicator of population bottlenecking only if the event is severe in strength or duration (Nei 1973). Small local effective population sizes are expected in isolated populations where gene flow is not powerful enough to counter genetic drift. The neutral model (Nei and Tajima 1981; Kimura 1983) predicts that small populations will be most subject to genetic drift and thus retain low haplotype diversity because genetic diversity is a direct function of

the effective population size (*Ne*) and mutation rate (μ). Nevertheless, nucleotide diversity in banded rail shows a decline with respect to the colonisation of most isolated archipelagos and recently established populations. This measure is a more sensitive indicator of changes in population size because rare alleles can be lost easily (Nei 1973). The loss of alleles may be due to population bottlenecking during range expansion to the more geographically isolated islands. This effect on nucleotide diversity therefore affects genetic structure, which is geographically distributed among archipelagos. In banded rails genetic diversity decreased and genetic differentiation (F_{ST}) increased as the colonisation events separating populations increased, regardless of high mobility.

For an island population to become isolated and to diverge, the island must first be colonized, and this presents an apparent paradox. Dispersal among islands must be frequent enough to enable colonisation, but not so common that the resulting gene flow retards speciation, since even moderate levels of gene flow have been shown to prevent divergence (Bolnick and Nosil 2007). A finding that divergence has occurred despite gene flow indicates that natural selection is driving divergence or alternatively, different rates, shifts and routes of gene flow are operating (Diamond et al. 1976; Mayr and Diamond 2001). If colonisation of islands is episodic then genetic drift in relatively small isolated populations is, over time, sufficient to accumulate differences. However, when the species appears to be widespread, gene flow among potential founder populations might be high, unless range expansion is not constant through time or the direction changes from time to time. The program IMa uses a demographic model for statistical analyses of movement of genes between populations, population sizes and time of population splitting (Hey and Nielsen 2007; Strasburg and Rieseberg 2010). Coalescent analysis allowed different levels of gene flow among archipelagos to be distinguished (Figure 5, Table S2). The unexpectedly large 90% HPD intervals, along with significant negative Fu's *Fs* values and significant positive R_2 values, suggest that the banded rail has recently expanded its range in Australia, Vanuatu, New Zealand and Bismarck. This leads to significant uncertainty in gene flow estimates because recent range expansions will mimic high rates of gene flow between distant populations (Slatkin 1993) and these results then are less clear than population sizes and splitting times, not only in terms of wider HPD intervals for parameter estimates but also in terms of sensitivity to prior distributions (Hey 2010). Nevertheless, my estimations

show a high level of gene flow and quite similar divergence times among populations, indicating that strong selection must be the key to the repeated insular adaptive radiation leading to diversification and evolution within this group.

The evidence of genetic changes and divergence on populations are important to understand speciation process within a supertramp/great speciator lineage. Particularly, birds that colonize islands embark on sequential phases of range expansion and contraction with genetic consequences (Losos and Ricklefs 2009; Reznick and Ricklefs 2009). Divergence of populations that colonize new habitats can produce differences in morphology or some other characteristics that may involve speciation. A disposition towards high phenotypic plasticity might enable populations to persist in new habitats (Richards et al. 2006; Schrey et al. 2011) and adaptive radiations are commonly associated with diversification in sparsely occupied ecological space, as may be typical during initial colonisation of remote islands. Evolution of ecological and phenotypic diversity within a rapidly multiplying lineage occurs when a single ancestor diverges into a host of species that use a variety of environments and differ in traits used to exploit those environments. The widespread and volant banded rail lives mainly in lowland habitats but several of its flightless closely related counterparts (e.g. the weka, *G. australis*, the Lord Howe woodhen, *G. sylvestris*) primarily use forested environments at higher elevations. These species provide a snapshot of the speciation process, leading from the initial occupation of open, lowland habitats, to expansion into forest and upland environments by adapting to island interiors. The flightless species are necessarily better suited in habits with specializations features associated to those environments than their forebears. Divergence operates by reducing ecological similarity whilst the ancestral colonist is isolated in small populations that are susceptible to local extinction. Old colonisations of banded rail were probably displaced by new colonisations into the forest where they evolved into flightless island endemics. These colonisations were decreasing and locally extinct and replaced by the flightless species and leaving only sporadic relict populations. This pattern is concordant with the appearance of the banded rail as the most derivative species within the *Gallirallus* tree phylogeny (Trewick 1997; Kirchman 2009; Trewick and Gibb 2010; Kirchman 2012). Overall, asymmetrical dispersion has meant that the flying lineage repeatedly established island populations which may lead to insular flightless species and replaced their progenitor.

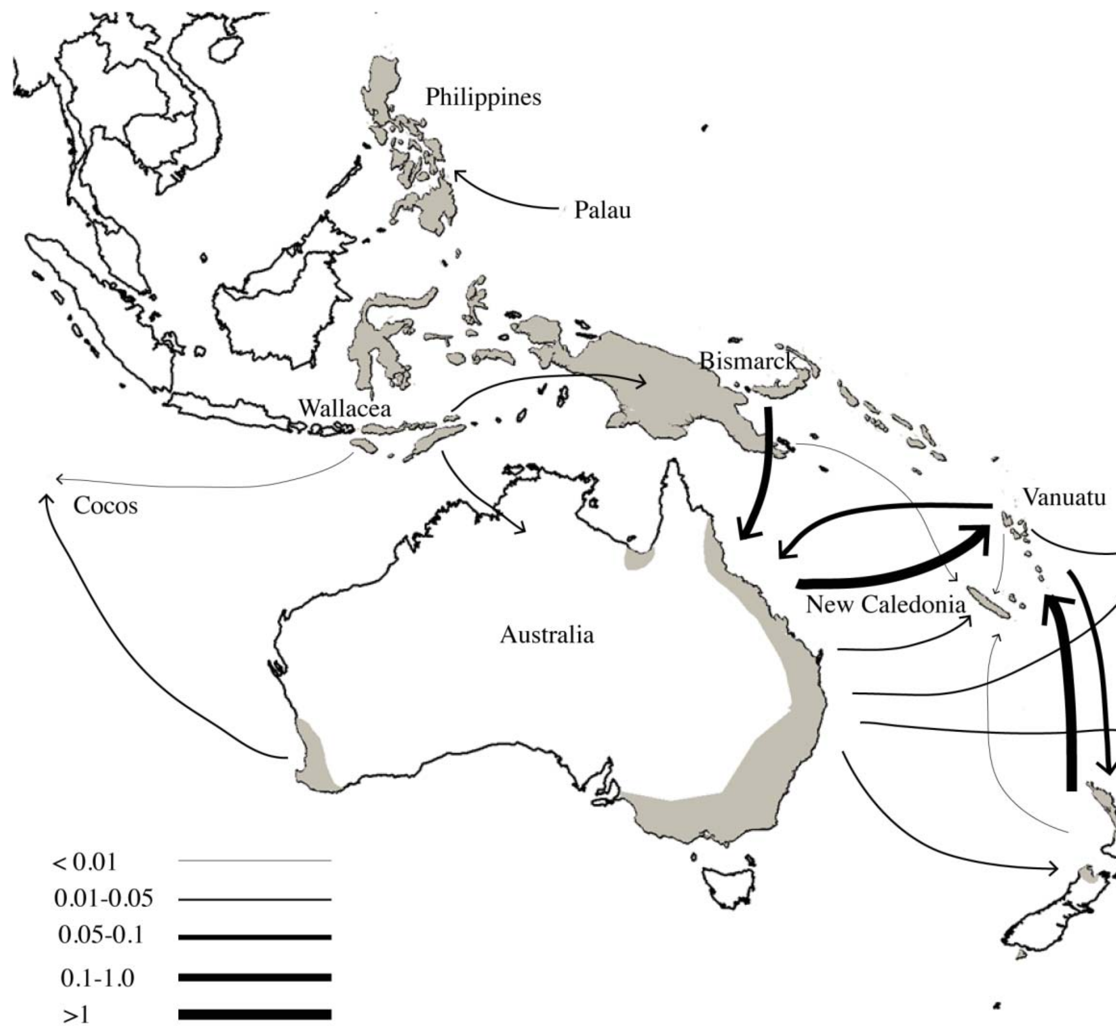

Figure 5. Map showing the likely direction of dispersal and colonisation events of banded rail in Oceania based on IMa sim
dispersion and the relative thickness is scaled to the extent of gene flow between sampled archipelagos.

Multiple step–wise colonisation events of the banded rail in Oceania are consistent with the assumptions of traditional stepping stone model of divergence. Indeed, the evidence suggests that the genetic differentiation is the result of subsequent colonisations of closely approximate demes by large numbers of individuals with Australia as the main intermediate source for dispersal to other islands. Several types of evidences suggest this conclusion. First, Australia shares haplotypes from five different archipelagos. Second, haplotypes whose distributions are distantly separated in Wallacea and Samoa occur together in Australia. Third, more recently established and isolated populations from Samoa and Cocos archipelagos have lower genetic variation than other populations close to Australia that are less prone to the mixture of haplotypes from multiple sources. Kirchman (2009) suggested that the banded rail expanded range out of the Philippines during the Last Glacial Maximum (LGM) about 20000 years ago. My estimations of divergence times are somewhat concordant with this timing (Table S2). During this period Australia was connected with New Guinea and Tasmania (Chabangborn et al. 2014). Likely, the Australian population of banded rail was established with the incursion of the northwestern populations from Wallacea and Bismarck archipelagos. The colonisation pattern is in accordance with a postglacial range expansion into New Zealand from a single glacial refugium (Australia). The network supports this finding because there are no clear subclades with numerous substitutions along branches connecting each of the subclades as expected if there were multiple refugial lineages (Reeves et al. 2008; Batalha-Filho et al. 2012; Klütsch et al. 2012; Zigouris et al. 2013). The step–wise mutation pattern of the banded rail provides evidence of the long–distance dispersal capabilities with most clinal variation of morphological subspecies derived from Australia (Schodde and Naurois 1982), which indicate shared ancestry.

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Table S1. Locality, museum voucher numbers, type of tissue, haplotype numbers, and GenBank accession numbers o
numbers from 1 to 38 have GenBank ID from Kirchman (2009). Acronyms for museums are: ANWC (Australian N
NMNZ (Te Papa Museum, New Zealand), UWBM (Burke Museum of Natural History and Culture, USA), MUNZ
New Zealand), EBU (Evolutionary Biology Unit at the Australian Museum, Australia), WAM (Western Australia Mus

Locality	Museum voucher	Type of tissue	Haplotype	Gen
New Caledonia	ANWC 19211	Muscle	1	
Vorovoro island, Fiji	NMNZ OR.020582	Toe pad	9	
Vorovoro island, Fiji	NMNZ OR.020581	Toe pad	40	
Vatulele island, Fiji	NMNZ OR.019554	Toe pad	9	
Vatulele island, Fiji	NMNZ OR.019553	Toe pad	15	
Ono island, Fiji	NMNZ OR.020653	Toe pad	9	
Ono island, Fiji	NMNZ OR.020652	Toe pad	15	
Taveuni island, Fiji	NMNZ OR.017838	Toe pad	2	
Taveuni island, Fiji	NMNZ OR.017841	Toe pad	2	
Taveuni island, Fiji	NMNZ OR.017839	Toe pad	43	
Upolu island, Samoa	NMNZ OR.019205	Toe pad	1	
Nofoa island, Samoa	NMNZ OR.027180	Toe pad	40	
Tutuila, American Samoa	UWBM 89687	Muscle	47	
Niue island	NMNZ OR.017393	Toe pad	1	
Niue island	NMNZ OR.017762	Toe pad	1	
Niue island	NMNZ OR.017763	Toe pad	1	
Northland, New Zealand	NA	Feathers	15	
Northland, New Zealand	NMNZ OR.018153	Toe pad	15	
Northland, New Zealand	NMNZ OR.018155	Toe pad	15	

Northland, New Zealand	NMNZ OR.022344	Toe pad	40
Northland, New Zealand	MUNZ12772	Muscle	44
Whakatane, New Zealand	NMNZ OR.028058	Toe pad	40
Great Barrier island, New Zealand	MUNZ12792	Muscle	26
Great Barrier island, New Zealand	MUNZ12793	Muscle	26
Great Barrier island, New Zealand	MUNZ12794	Muscle	26
Great Barrier island, New Zealand	MUNZ12795	Muscle	26
Great Barrier island, New Zealand	MUNZ12796	Muscle	40
Kundy Island, New Zealand	NMNZ OR.028830	Toe pad	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	40
Golden Bay, New Zealand	NA	Faeces	41
Golden Bay, New Zealand	NA	Faeces	48
NSW, Australia	EBU 39560	Muscle	15
NSW, Australia	ANWC 43303	Muscle	40

NSW, Australia	ANWC 34178	Muscle	45
NSW, Australia	EBU 39536	Muscle	46
Lord Howe island, Australia	EBU 38482	Muscle	41
SA, Australia	EBU 45507	Muscle	24
QLD, Australia	ANWC 44301	Muscle	22
QLD, Australia	ANWC 10622	Muscle	22
QLD, Australia	ANWC 32326	Muscle	26
WA, Australia	WAM 34289	Muscle	20
WA, Australia	WAM 33896	Muscle	20
WA, Australia	WAM 35742	Muscle	24
Cocos island, Australia	NA	Blood	24
Cocos island, Australia	NA	Blood	24
Cocos island, Australia	NA	Blood	24
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
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Cocos island, Australia	NA	Blood	39
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Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	39
Cocos island, Australia	NA	Blood	42
Cocos island, Australia	NA	Blood	42
Sumba island, Indonesia	WAN 22849	Muscle	20
Sumba island, Indonesia	WAN 22850	Muscle	24
Sumba island, Indonesia	WAN 23475	Muscle	49

Table S2. Estimates obtained from IMa for the population size parameter theta (*Ne*), gene flow (*Nm*) and divergence times (*t*) and 90% highest posterior density (HPD) intervals for archipelagos dataset, where Phil = Philippines, Aus = Australia, NCal = New Caledonia, and NZ = New Zealand.

Samples	Parameters		
	HiPt	90% HPD	
		Lower	Upper
Ne			
Philippines	152190	56767	596573
Palau	86543	28384	328585
Wallacea	133047	84073	543887
Bismarck	160592	92492	327243
Australia	250696	143640	749008
New Zealand	135046	86086	773961
New Caledonia	22724	5833	155063
Vanuatu	133179	65145	588873
Fiji	53667	21099	258422
Samoa	47720	16284	249926
Cocos	17685	2449	442674
Nm			
Palau→Phil	0.037	0.014	168.480
Wallacea→Bismarck	0.018	0.011	65.436
Wallacea→Cocos	0.009	0.001	431.126
Wallacea→Aus	0.029	0.015	292.903
Bismarck→NCal	0.005	0.002	101.282
Bismarck→Aus	0.131	0.066	763.197
Aus→NZ	0.016	0.005	353.506
Aus→NCal	0.036	0.019	295.725
Aus→Vanuatu	2.08	0.03	396.59
Aus→Fiji	0.039	0.011	304.588

Aus→Samoa	0.014	0.006	188.161
Aus→Cocos	0.024	0.001	212.145
NZ→NCal	0.007	0.002	88.289
NZ→Vanuatu	2.780	0.049	378.400
NZ→Fiji	0.014	0.003	241.613
NZ→Samoa	0.022	0.009	212.336
Vanuatu→Aus	0.098	0.056	472.640
Vanuatu→NZ	0.066	0.042	639.140
Vanuatu→NCal	0.008	0.003	57.435
Vanuatu→Fiji	0.044	0.025	695.814
Fiji→Samoa	0.004	0.001	40.568
Samoa→Fiji	0.017	0.007	167.787
t			
Palau–Phil	46059	6650	492365
Wallacea–Bismarck	42488	2833	137069
Wallacea–Cocos	20320	74	141946
Wallacea–Aus	28227	4581	295419
Bismarck–NCal	49212	3990	221823
Bismarck–Aus	46552	19951	105369
Aus–NZ	21798	123	246182
Aus–NCal	38867	3695	198473
Aus–Vanuatu	42709	11084	175714
Aus–Fiji	33842	3103	295123
Aus–Samoa	24975	1034	294532
Aus–Cocos	24877	2217	492365
NZ–NCal	50862	6773	246182
NZ–Vanuatu	43300	8128	221232
NZ–Fiji	40025	123	193966
NZ–Samoa	33128	123	209729

Vanuatu–NCal	47167	5049	191502
Vanuatu–Fiji	37315	5296	164655
Fiji–Samoa	25000	123	237562

Discussion

Processes leading to changes in biological diversity vary in time, space and among groups of organisms. Our current knowledge of the evolution and diversification of birds has improved in the last decades thanks to two complementary sources of information: fossils and genes (van Tuinen 2002). A major source of data for studying diversification processes, including speciation/origination, extinction, migration, and population changes, is the fossil record. However, evolutionary changes are mostly represented by few fossils that are widely separated in space and time. New insights into evolution have come particularly from molecular data because morphology has often misled our inferences of modern bird relationships (Hackett et al. 2008). Rapid diversification can produce many divergent morphological groups that are closely related while the recognition of deep radiations that are not closely related may be confounded by convergence of traits. Convergence along with unique characters, in some cases makes it more difficult to place fossil taxa with precise certainty in phylogenies, especially when they are fragmentary. This problem has influenced inferences about the timing of avian divergences (Ericson et al. 2006; Hackett et al. 2008) among other important questions.

Understanding the evolution of birds and the distribution of its diversity depends on having accurate phylogenetic information to provide historical, ecological and geographical contexts (Jetz et al. 2012; Ricklefs and Pagel 2012). However, to achieve this, and to obtain a detailed picture of the evolutionary history of birds, an extensive sampling at different taxonomic levels is necessary. My research investigated ecological and evolutionary processes generating and maintaining biodiversity across spatial and temporal scales at different systematic levels, with an empirical focus on rail diversity. I have concentrated on reconstructing family and genus level phylogenies to provide a framework in which to explore microevolutionary processes. I complemented this by exploring species and populations to infer the mechanisms operating in speciation. By taking these approaches, I generated robust estimations of origin and diversification of rails and hypothesized the relative contributions of selection, migration, phenotype, behaviour, ecology, geology and climate on variation and distribution of the extant rail biological diversity.

Origin of rails

The family Rallidae is a complex group to study because of the apparent paradox of sedentary mode and colonizing capacity that often results in flightless insular species. Previous studies have addressed the question of the origin of the Gruiform order (Fain et al. 2007; Houde 2009), that includes Rallidae, and the biogeography and evolution of some rail lineages have been recently considered (Trewick 1997a; Kirchman 2009; Kirchman 2012; Ruan et al. 2012). However, identification of the basal split and diversification of the rails has remained uncertain because of differences in the estimated dates and approaches used (Ericson et al. 2006; Brown et al. 2007; Fain et al. 2007; Houde 2009). My analysis suggests (Chapter 1) that the living rail clade originated during the Middle/Late Eocene (~40.5 Mya) and high diversification may reflect processes that operate in both space and time. The remarkable capacity of the rails to adapt to a wide variety of habitats has perhaps favoured its diversification dynamics in a temporal context. For instance, during the Eocene/Oligocene transition a climatic crisis was characterized by a drop in the mean annual temperature and changes in vegetation (Prothero 1994; Zachos et al. 2001) with a gradual faunal turnover (Prothero 1989; Ivany et al. 2000). This event created an important biotic reorganization and many modern families of birds first appeared and diversified in this time frame (Blondel and Mourer-Chauviré 1998; Lindow and Dyke 2006; van Tuinen et al. 2006). In particular, adaptation to more open areas and grasslands seems to have been favoured (Collison and Hooker 1987; Prothero 1989; Prothero 1994; Hooker and Collinson 2012). Several ecological niches were available during that time that may have helped to increase the diversification of species related to forest-dwelling and other land ecological types.

The estimated time of rail origin disagrees with the current fossil record but in a way that allows evolutionary consistency. The phylogenetic age is older than current fossils. The earliest rail fossils currently available come from the latest Eocene–earliest Oligocene deposits of Europe (Olson 1977; Mayr and Smith 2001). Nevertheless, some fossil genera tentatively attributed to Rallidae (e.g. *Palaeorallus*, *Eocrex* and *Fulicaletornis*) are from Early Eocene time in North America (Wetmore 1931; Olson 1977) and the rail-like taxa of the genus *Songzia* are from the Early Eocene in China (Hou 1990; Wang et al. 2012).

Unfortunately, the precise placement of many of these fossils within Rallidae is equivocal (Mayr 2005, 2009) because of the fragmentary nature of the specimens and many missing diagnosable characters. As with the continuing discovery of Cretaceous bird fossils (e.g. Stidham 1998; Clarke et al. 2005; Fountaine et al. 2005; Brocklehurst et al. 2012) it is likely that better rail specimens will be forthcoming. The apparent conflict between fossil and molecular dates is most simply explained by the incomplete fossil record. Paleontological studies rely on fossils to directly infer minimal divergence times and molecule-based studies rely on fossils to calibrate chronograms with good time and morphological constraints. New fossil records in combination with accurate phylogenetic relationships will likely impact both types of studies. My analysis utilised well-constrained fossils from outside the Rallidae and Gruiformes which is preferable for the calibration of internal nodes.

Systematic relationships, taxonomy and character evolution in rails

Embedding species in the appropriate phylogenetic context is the way to determine the direction of evolutionary trends and add rigor into evolutionary hypothesis. When I started this research there was uncertainty about the systematic relationships among genera and species within Rallidae. My proposed molecular phylogeny of genera (Chapter 2) reconciles a seemingly arbitrary taxonomic treatment that has developed since Linnaeus (1758), and in many respects is consistent with the simpler earlier proposals/hypothesis. My phylogenetic analyses produced many well-resolved nodes that underpin taxonomic and systematic changes. These analyses confirm that *Sarothrura* and *Canirallus*, previously placed in Rallidae, have affinities outside the family and this placing corroborates the proposal of Slikas et al. (2002), Fain et al. (2007) and Hackett et al. (2008) who placed this lineage in its own family, Sarothruridae. Within the monophyletic radiation of Rallidae I recognized eight major clades that render the current treatment of some genera as either para– or polyphyletic. The present genus *Porzana* is polyphyletic with species found in "*Fulica*", "*Aramides*", "*Laterallus*" and core "*Porzana*" clades (see also Livezey 1998; Slikas et al. 2002). The Sora crake (*Porzana carolina*), Spotted crake (*P. porzana*) and Australian spotted crake (*P. fluminea*) are placed with the Spot–flanked gallinule (*Gallinula melanops*), Black-tailed native–hen (*G. ventralis*) and Tasmanian native–hen (*G. mortierii*)

within the "*Fulica*" clade; while the South American species Ash–throated crake (*Porzana albicollis*) is more closely allied with another endemic South American genus (*Neocrex*) within the "*Aramides*" clade. Two other American species included in this study, the Dot-winged crake (*P. spiloptera*) and the Yellow–breasted crake (*P. flaviventer*) are more closely related to species also in the American genus *Laterallus*. The Yellow-breasted crake is closely related to the Grey-breasted crake (*Laterallus exilis*) and the White-throated crake (*L. albigularis*), while the Dot-winged crake (*P. spiloptera*) is closely related to the Black rail (*L. jamaicensis*) within the "*Laterallus*" clade. The rest of the species within the genus *Porzana* are clustered with the Black crake (*Amaurornis flavirostra*) and the Brown crake (*A. akool*) in the "*Porzana*" clade.

Previous classifications placed the Spot-flanked gallinule in the monotypic genus *Porphyriops* (Vieillot 1819), but put the Black-tailed native–hen and Tasmanian native–hen within the genus *Tribonyx* (Gould 1837). Morphological characters (short toes, lack of white in the under tail coverts, long tails and short frontal shields) unequivocally supported the placement of Black-tailed native–hen and Tasmanian native–hen apart from the other gallinules (Olson 1973a). The Spot-flanked gallinule along with the Red–fronted coot (*Fulica rufrifrons*) have been suggested as bridging species between the coots and the gallinules (Olson 1973a). The emergence of the Red–fronted coot lineage from the base of the genus *Fulica* is consistent with the presence of distinctly narrow lateral membranes of the toes in this species compared to other coots (Olson 1973a).

Olson (1973a) proposed a close relationship among the Neotropical Uniform crake (*Amaurolimnas concolor*) and *Aramides* species based on similarities in habitat occupancy and colour, and this is confirmed in my analysis. My results also showed the grouping of the Russet–crowned crake (*Anurolimnas viridis*) and *Laterallus viridis*. The data of the former species was obtained from GenBank while the latter came from this study by tissues obtained from the Emílio Goeldi and Universidade de São Paulo Museums, both from Brazil. The generic name (*Laterallus*) of the samples from both museums in Brazil was retained to corroborate the insertion in *Anurolimnas* as suggested by Olson (1973a). The Southeast Asian species White–breasted water–hen (*Amaurornis phoenicurus*) and the

Australasian species Pale–vented bush–hen (*A. moluccana*) were found to be closely related to the Watercock (*Gallicrex cinerea*) confirming the morphological and molecular similarity found by Olson (1973a) and Ruan (2012), respectively. The placement of the African Nkulengu rail (*Himantornis haematopus*) obtained in my analysis is novel and surprising given traditional treatments. This species has been considered the most "primitive" in Rallidae and traditionally placed in its own subfamily, Himantornithinae (Olson 1973a; Ripley 1977; Livezey 1998). My results contradict the placement of Nkulengu rail outside of "Rallinae" (the remaining genera in Rallidae), placing it within core rails (i.e. discard Subfamilies) and allied to another aberrant species, the New Guinea flightless rail (*Megacrex inepta*).

My multilocus phylogenetic study recovered the close relationship of *Lewinia* species and the Slaty–breasted rail (*Gallirallus striatus*) (Olson 1973a; Kirchman 2012) and these species appear to be allied to *Aramidopsis, Crex* and *Dryolimnas* ("*Rallus*" clade). Consistent with previous findings (Kirchman 2012), I also identified the Chestnut rail (*Eulabeornis castaneoventris*) and the Invisible rail (*Habroptila wallaci*) as part of *Gallirallus* group. The sampling also allowed inference of the phylogenetic position of the near threatened Woodford's rail (*Nesoclopeus woodfordi*) and the extinct Giant Chatham Island Hawkins's rail (*Diaphorapteryx hawkinsi*) as part of the *Gallirallus* radiation in Oceania. Kirchman (2012) included the species of *Lewinia* and *Aramidopsis* within the *Gallirallus sensu lato* group, which is contradictory to the evidence presented here.

Misinterpretation or wrong estimation of phylogenetic relationships can affect inferences of species traits. Methods that estimate ancestral-states also have implicit biases caused by sampling dependence. However, inferences obtained in this research have important implications for the evolutionary ecology of rails because they link the evolution of organismal traits with geographic range and ecology. Reconstruction of the history of the frontal shield clearly suggests multiple independent development of this trait within "*Gallicrex*", "*Fulica*" and *Porphyrio* clades around 15 Mya. The presence of a frontal shield is may be plesiomorphic in *Porphyrio* and "*Fulica*" and associated with mate recognition (Gordon 1951; Craig 1977; Crowley and Magrath 2004). Contrary to previous

ideas, forest is not supported as the ancestral habitat of the family and my results suggest that wetlands are the most probable ancestral state for Rallidae. At the very least, wetland appears to be the predominant habitat among extant taxa. Nevertheless, any of the habitats, except islands, could be the ancestral state for *Gallirallus*. This reflects the regional importance of *Gallirallus* in the “exploded continent” of Oceania which is related to other traits and processes.

Large birds are found in volant and flightless lineages that otherwise also include medium size birds, whilst small birds are mostly in the “*Laterallus*” and “*Porzana*” clades. However, the inference that large size is ancestral in *Gallirallus* is likely to be an artefact of extinction; the weka (*G. australis*) is a survivor on a long branch. Ancestral state analysis implies that flight has re-evolved from flightless ancestors, but other evidence shows this to be highly unlikely (Olson 1973a; Trewick 1997a). This reveals once again a general problem with ancestral state reconstruction (Cunningham et al. 1998; Omland 1999; Crisp and Cook 2005; Ekman et al. 2008). The tendency for the loss of flight in *Gallirallus* may lie in their ontogenetic predisposition interacting with insular distribution that exposes populations to novel selective regimes (Olson 1973b; McCall et al. 1998; McNab and Ellis 2006). In particular, a trade-off between energy conservation and predator avoidance is apparent (McNab 1994; McNab and Ellis 2006). A pattern of directional asymmetry in dispersal is the best explanation for the apparent evolution of flight in banded rail (*Gallirallus philippensis*) which emerges as a more derivative species within *Gallirallus*. In fact, the flying lineage must have repeatedly established island populations that each give rise to insular flightless species that locally replaced their flying founder (Cook and Crisp 2005; Crisp et al. 2011).

Diversification and biogeography of rails

Dispersal ability and ecological variety have allowed diversification and global retention of lineage diversity in multiple clades within Rallidae (Olson 1973a; Ripley 1977). The result is a biogeographic pattern involving representation of most genera (clades) in all habitable continents rather than generic radiations being associated with particular geographic regions. Bursts of lineage diversification span across archipelagos and continents. Rapid

and shallow speciation within some clades is found due to radiation on islands after colonisation (e.g. *Gallirallus*) and habitat patches in continental landscapes (e.g. *Fulica*). But other processes are also implicated in Rallidae diversification. My study tracks early episodes of diversification over 30 Mya. Old clades are important for rail diversity and diversification is probably related to occupancy of novel habitats propelled by major climatic shifts (Zachos et al. 2001; Böhme 2003; Kürschner et al. 2008; Tolley et al. 2008). The molecular phylogeny suggests differing rates of diversification with "*Rallus*", "*Fulica*" and "*Laterallus*". These clades retain most of the lineage diversity within Rallidae compared to other clades, contributing to the accumulation of diversity rather than a short-lived burst of speciation. Phenotypic differentiation associated principally with sexual selection (e.g. frontal shield) and morphological innovations (e.g. flightlessness) are important in lineage diversification. The frontal shield in *Porphyrio* and "*Fulica*" is known to be associated with social interactions, such as status, dominance, mate recognition and choice (Gordon 1951; Craig 1977; Crowley and Magrath 2004; Dey et al. 2014). It is possible that other characters in other clades are involved in sexual selection and speciation.

Taking into account available information (Trewick 1997a; Slikas et al. 2002; Kirchman 2012; Ruan et al. 2012) and based on my results, a reduced number of genera provides a clearer hypothesis of how the diversity within Rallidae is distributed, where each clade may have originated, and possible directions of range expansion. Many of the currently recognized genera are embedded within widespread genera that are more morphologically homogeneous. "*Fulica*" and "*Rallus*" clades have worldwide distributions and likely originated in the Americas. "*Porzana*" occurs in Europe, Africa, Asia and Oceania while "*Gallicrex*" is found in the latter three, but both have a possible African origin. An African origin might also be ascribed to *Porphyrio* clade (discussed in chapter 3) even though this requires long distance dispersal events into the Americas and Europe, Asia and Oceania. *Rallina* is found across Asia and Oceania along with *Gymnocrex* (Olson 1973a). "*Laterallus*" and "*Aramides*" are endemic to the Americas along with *Micropygia* and *Cyanolimnas* (Olson 1973a), while *Atlantisia* and *Rougetius* (Olson 1973a) are found in Africa or on nearby islands. However, I note that clade representation around the world is often dependent on one or a few supertramp lineages such as the purple swamphen

(*Porphyrio porphyrio*) within *Porphyrio*, the banded rail (*Gallirallus philippensis*) within "*Rallus*", the common coot (*Fulica atra*) and the common moorhen (*Gallinula chloropus*) within "*Fulica*", and Baillon's crake (*Porzana pusilla*) and the spotless crake (*Porzana tabuensis*) within "*Porzana*". These species are widespread across several thousands of kilometres and present in high abundance in some local populations (Diamond 1982; Mayr and Diamond 2001; Sullivan et al. 2009; BirdLife International 2014). The prominent role of dispersal as an important life-history trait among these species has helped a rapid radiation in archipelagos, mainly of the south west Pacific (Diamond 1977; Trewick 1996, 1997a; Trewick 1997b; Mayr and Diamond 2001). High dispersal ability, huge abundance and rapid adaptive species (Olson 1973a, b; Ripley 1977; Ripley and Beehler 1985; Kirchman 2009), leads to speciose and widespread genera (Darwin 1859). The occurrence of species with remarkably different ecology and dispersal ability in the same clade likely reflects rapid shifts in these attributes over evolutionary time (Andersen et al. 2014).

For instance, the phylogenetic analyses and molecular dating in *Porphyrio* (Chapter 3) support independent and temporally non-overlapping colonisation events within this clade. However, it is necessary to note that these interpretations are based on surviving lineages only and other colonisations are extinct (Steadman 1988; Steadman et al. 1999; Steadman 2006) but known from fossils or leave no trace at all. The most likely area of origin of *Porphyrio* is Africa with colonisation occurring westwards into the Americas and several via the North-East (Europe, Asia and Oceania) during the Miocene and Pleistocene time. The oldest split among the currently recognized *Porphyrio porphyrio* lineage occurred in late Miocene time around 6 Mya. Despite the limited use of flight of these *Porphyrio* subspecies/species, they have dispersed, colonized and established populations multiple times across large open expanses of water favoured perhaps by sea-level changes associated with glaciations in the Pleistocene (Voris 2000; Jønsson et al. 2010; Lohman et al. 2011; Condamine et al. 2013; Irestedt et al. 2013). *Porphyrio p. melanotus* has become widespread in Australasia within the last 600 thousand years but bone deposits show a very recent arrival on some remote islands (Millener 1981; Taylor 1998; Steadman 2006), including New Zealand about 300 years ago (after Polynesian settlement) (Trewick and Worthy 2001). This dating is much more recent than the estimated divergence of swamphen

and Takahe lineages around 2 Mya and indicates that is not the lineage that gave rise to the flightless insular species of New Zealand.

Within the geographical region of Australia and New Zealand, the distribution of genetic variability in *P. p. melanotus* indicates genetic structured populations. Colonisation of this species/subspecies may have started in the Wallacea region and been favoured in the Indo-Pacific region by sea-level changes and land-bridge formations during the Pleistocene creating the allopatric pattern of distribution currently shown (Voris 2000; Hall 2009; Wurster et al. 2010; Lohman et al. 2011). Genetic diversity in Australia is higher than across the Tasman Sea in New Zealand indicating an influence of distribution due to persistence of habitat rather than geographic distance. Within this region, *P. p. melanotus* shows prominent population differentiation in plumage colour pattern in populations from south Western Australia (the current nominate subspecies *P. p. bellus* has a brighter blue colour in the breast and throat). Variation in colour, size and other traits are also seen in other subspecies within the *melanotus* group (Ripley 1977). The mismatch between plumage and neutral population genetics suggests that differentiation in colour and other traits have arisen rapidly in *Porphyrio* and are subject to selection of the genes in a local environment (Mayr 1954; Nosil et al. 2009; Feder et al. 2012) or stochastic genetic drift (Clegg et al. 2002a; Clegg et al. 2002b). The lack of sorting at the BFG–7 locus hints that fixed plumage colour differences among populations are not the result of drift but are perhaps under sexual selection.

In order to understand how the current rail diversity has evolved it is necessary to estimate fine scale processes influencing population structure and speciation (Chapter 4). Genetic changes and divergence in populations are informative about the processes of speciation within a supertramp/great speciator lineage. Particularly, birds that colonize islands embark on sequential phases of range expansion and contraction with genetic consequences (Losos and Ricklefs 2009; Reznick and Ricklefs 2009). Divergence of populations that colonize new habitats can produce differences in morphology or some other characteristics that may involve speciation. A disposition towards high phenotypic plasticity might enable populations to persist in new places (Richards et al. 2006; Schrey et al. 2011) and adaptive

radiations are commonly associated with diversification in sparsely occupied ecological space, as may be typical during initial colonisation of remote islands. Evolution of ecological and phenotypic diversity within a rapidly multiplying lineage occurs when a single ancestor may diverge into a host of species that use a variety of environments and differ in traits used to exploit those environments. For instance, the widespread and volant banded rail (*Gallirallus philippensis*) lives mainly in lowland habitats but several of its flightless closely related counterparts (e.g. the weka, *G. australis*, the Lord Howe woodhen, *G. sylvestris*) primarily occupy forested environments at higher elevations. These species provide a snapshot of the speciation process, leading from the initial occupation of open, lowland habitats, to expansion into forested and upland environments by adapting to island interiors. Ecological speciation operates by reducing their ecological similarity whilst the ancestral colonist is isolated in small populations that are susceptible to local extinction. Old colonisations of banded rail were probably displaced by new colonisations into the forest where they evolved into flightless island endemics. These colonisations were decreasing and locally extinct and replaced by the flightless species leaving only sporadic relict populations. This pattern is concordant with the appearance of the banded rail as the most derivative species within the *Gallirallus* phylogeny (Trewick 1997a; Kirchman 2009; Trewick and Gibb 2010; Kirchman 2012). Asymmetrical dispersion has meant that the flying lineage has repeatedly established island populations that have each given rise to insular flightless species locally replaced by their progenitor.

Overall, the main focus of this thesis was to recognize the temporal and spatial scales of macro– and micro–evolution in a phylogenetic and population context using empirical studies in Rallidae. This study expands our knowledge on the causes, factors and processes of generation, variation and distribution of biodiversity on Earth. Naturally, my research leads to fertile opportunities for improving our understanding of both bird (and vertebrate) diversity in space and time and the processes shaping that biodiversity. For instance, the role of ecology has perhaps been underestimated in current biological research, reflecting a gap on the state of the art that must be filled to have a holistic appreciation. Exploring research in this field can provide further explanations of diversification, especially when incorporating species interactions using ecological network analyses and community

assembly or phylogenetic diversity (Webb et al. 2002; Rezende et al. 2007; McPeek 2008; Morlon et al. 2011; Morlon 2014). Another comprehensive area for exploration is the integration of population genetic and ecological data to model the influence of climate (and other environmental factors) on lineage evolution (Kozak et al. 2008). The association between environment, phenotype and genotype in response to natural selection will help us to understand the drivers of speciation and the first stages of spatial variation in biological diversity. Evaluation of evolutionary rates on latitudinal gradients related to individual growth rates, variance in fecundity, recruitment, dispersion among populations (with help of geopositional data and radio-telemetry) and a variety of biotic interactions, is a central topic in evolutionary science that should be a focus of forthcoming studies (Martin and McKay 2004; Cardillo et al. 2005; Mittelbach et al. 2007; Dowle et al. 2013). Assessing the role of species traits shaped by sexual selection at macro and micro–evolutionary scales, as well as different spatial scales, is a fascinating step toward resolving the evolutionary history of birds (Turelli et al. 2001). Matching patterns of spatial phenotypic variation, selection and population ecological data (species density and variability in habitat) are important to our understanding of adaptation, speciation (even in sympatry) and mechanisms responsible in evolution of reproductive isolation (Seehausen et al. 2008; Ricklefs 2010; Maan and Seehausen 2011). Similarly, exploring dynamic relationships in mate choice based on sensory signals, assessing the relative rates of evolution between recently divergent sister species and local/temporal variations in selective regimes (e.g. host–parasite coevolution) will provide fruitful foci for research to evaluate progress towards reproductive isolation and speciation (Seehausen et al. 2008; The Marie Curie SPECIATION Network 2012). Another subject area that provides a framework to elucidate the genetic basis of speciation is the study of Quantitative Trait Loci (QTL). Elucidating the genetic architecture and additive genetic variation using QTL is core to identify the location and mode of action of genes and genomic regions contributing in phenotypic variation/adaptation, selection and speciation (Orr 2001; Pavlicev et al. 2011; Robinson et al. 2013). These studies will help us to uncover the processes that have generated current biodiversity and understand speciation events placing particular emphasis on the evolutionary forces, ecological circumstances and genetic mechanisms that have led to the development of species.

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