Articles

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# Interisland gene flow among populations of the buff-banded rail (Aves: Rallidae) and its implications for insular endemism in Oceania

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Studies of fragmented habitat, such as island archipelagos, provide insights into the microevolutionary processes that drive early stages of diversification. Here, we examined genetic variation and gene flow among populations of the widespread buff-banded rail *Gallirallus philippensis* in Oceania to understand the factors that promote speciation associated within this bird lineage. We analysed mtDNA Control Region sequences and six microsatellite loci from a total of 152 individuals of buff-banded rail on islands and continental areas. We used a phylogeographic model-testing approach and a structured spatial design ranging from within to among archipelagoes in the south Pacific. Buff-banded rail populations in the Philippines archipelago and nearby Palau and Wallacea had high genetic diversity while those in geographically distant Australia showed lower variation. Other archipelagos sampled were found to have less genetic diversity and included haplotypes closely related to Wallacea (Bismarck, Vanuatu, New Caledonia) or Australia (New Zealand, Samoa, Fiji, Cocos Islands). Nucleotide diversity and allele frequency declined with degree of geographic isolation but haplotype diversity remained more even. However, both nucleotide and haplotype diversities were positively correlated with land area. Microsatellite data for a subset of locations showed moderate to high genetic differentiation and significant pairwise $F_{ST}$ despite a relatively high migration rate. Our results are mostly consistent with a model of abrupt genetic changes due to founder events with multiple dispersals into Australia from Wallacea and Bismarck. Australia has probably been the source of birds for islands in the Pacific. This is shown by decreasing genetic diversity and growing genetic differentiation when distances separating populations increased from Australia. A history of range expansion and divergent natural selection may help explain the existence of numerous sympatric *Gallirallus* island endemics.

Genetic exchange and ecological interchange are the mechanisms that maintain cohesion among populations occupying different habitat patches (Stockman and Bond 2007, Petit and Excoffier 2009). Gene flow between populations in disjunct habitat might continue but it can be overwhelmed by the effects of selection resulting in independent evolutionary trajectories of physically separated populations (Via 2012). Shifts in population genetic diversity associated with adaptive changes can also result from range expansion across a fragmented habitat matrix (Clegg et al. 2002). These dispersal events may be largely stochastic but establishment is likely to be linked with habitat preference and distribution of external biotic features (Edelaar et al. 2008, Edelaar and Bolnick 2012). Colonisation is then expected to be strongly influenced by the availability of niche space with few competitors (i.e. colonisation opportunity) and potential to exploit ecological opportunities (Diamond 1975).

Theory predicts that among habitat patches with abrupt boundaries, such as oceanic islands, rates of colonisation are mediated mostly by geographic distance, and populations on mainland or large islands are likely to be sources of migrants rather than recipients (MacArthur and Wilson 1967, but see Filardi and Moyle 2005, Bellemain and Ricklefs 2008, Jonsson and Holt 2015). Colonist populations generally have low genetic diversity but gene flow from multiple independent dispersions, mix of individuals from geographically and genetically distinct origins and recombination between divergent genomes can result in an increase of genetic variability (Young et al. 1996, Grant et al. 2001, Kolbe et al. 2004). Nevertheless, the population genetic changes caused by founder events can be estimated by comparison of genetic variation in conspecific populations despite a lack of information of the timing and sequence on colonisations.

Islands of the western Pacific Ocean have long stimulated thought about avian speciation and biogeography and are recognized as natural laboratories of evolution (Wallace 1876, Diamond 1977, Mayr and Diamond 2001, Filardi

and Moyle 2005, Moyle et al. 2009). Physical isolation is expected to play an important role in biological diversification of oceanic islands, but the occurrence of some bird species across archipelagos is testimony to dispersal ability that could counteract the influence of geographical barriers and natural selection (Trewick and Gibb 2010, Goldberg et al. 2011). The presence of insular endemic species that evolved over short periods of isolation on the same islands as widespread close relatives that retained their dispersal capacity suggests a dynamic interplay between rates of gene flow and natural selection (Clegg 2010, Garcia-R et al. 2014).

The bird genus *Gallirallus* (Rallidae) comprises widespread volant and endemic flightless species on islands in the western Pacific (Fig. 1A). Although superficial similarity of insular species hints at their sharing flightless ancestry (Beauchamp 1989) and parsimony based ancestral state reconstruction of some traits supports this inference (Garcia-R et al. 2014), biology and landscape history renders it unlikely. The islands occupied by flightless species are widely spaced and of varying age and geology (Neall and Trewick 2008), and phylogenetic analysis of some of the many endemics and all of the volant species supports numerous independent origins of insular flightless taxa (Garcia-R et al. 2014, 2016), several of which have been placed in separate genera (e.g. *Nesoclopeus*, *Habroptila*, *Diaphorapteryx*) (Fig. 1B). Diversification of *Gallirallus* appears therefore to be primarily the result of the repeated evolution of flightless species on islands such that they look different from their flying relatives but similar to other flightless species (Diamond 1991, Trewick 1997b, 2011). While it is obvious that flightlessness reduces the likelihood of further gene flow among island populations, this condition must first evolve in the face of potential gene flow among populations of the progenitor. A temporally uneven rate of gene flow, the genetic effects of founder events, genetic drift and/or intense local selection would be needed for the observed outcome (Trewick 1997a).

The most geographically widespread of the *Gallirallus* group is the volant buff-banded rail *Gallirallus philippensis,* which is distributed from the Philippines (~ 18° latitude North) in southeast Asia to subantarctic Macquarie Island in the Southern Ocean (~ 55° latitude South) in Oceania, and from the Cocos Islands (~ 97° longitude East) in the Indian Ocean to Samoa (~ 172° longitude West) in the Pacific Ocean (Fig. 2). Although the broad distribution of this species over large bodies of water shows a propensity for dispersal and colonisation among islands, their dispersal habits are not well known. As many as 20 subspecies differ mainly in plumage colours and occupy separate on different archipelagos (Ripley 1977, Schodde and Naurois 1982, Elliott 1987, Taylor 1998). This implies dispersal is not ubiquitous and predicts spatial genetic structure caused by limited gene flow. The extensive but patchy geographic range with numerous regionally differentiated populations and subspecies make it a suitable model for integration of ecological and historical approaches using fine-scale phylogeographic analyses.

Understanding modern population structure in the buff-banded rail provides valuable insights into historic processes that could in turn help explain insular speciation of *Gallirallus* in the southwest Pacific. We examined patterns of genetic variation among populations to make inferences about the colonisation process. We describe genetic diversity of the wide-ranging buff-banded rail by applying traditional population genetic analyses and coalescent models so as to allow estimation of migration and colonisation history. Specifically we quantified genetic differentiation within and among populations of buff-banded rail in Oceania and the Philippines, determined the genetic structure across its geographical distribution, and discerned pathways of colonisation and patterns of gene flow among geographically structured groupings to address the following questions: 1) how is regional/subspecies genetic diversity of buff-banded rail distributed across its geographical range? 2) Is archipelagic population genetic structure consistent with current subspecies taxonomy? 3) Does geographical spatial distribution explain the inferred rates of gene flow? 4) Can population genetic analyses in buff-banded rail help to explain the evolution of insular endemics in *Gallirallus*?

## Material and methods

### Sampling and laboratory techniques

Toe pad and muscle tissue of *Gallirallus philippensis* were obtained from museum collections while feathers, faeces or blood were collected in the field and preserved in ethanol after success (feathers and blood) or failure (faeces) capturing birds using live trapping (Supplementary material Appendix 1, Table A1). Ancient DNA (aDNA) extraction from toe pads was carried out in a dedicated ancient DNA laboratory at Ecology Group, Massey Univ. (<http://evolves.massey.ac.nz/DNA_Toolkit.htm>) using either standard phenol-chloroform or the Qiamp DNA Minikit (Qiagen). DNA was obtained from blood, muscle and feathers using the Qiagen QIAamp tissue kit (Qiagen) and DNA from faeces was extracted using either a phenol-chloroform method or the Nucleospin soil kit (Macherey–Nagel).

### Mitochondrial DNA analyses

We generated mitochondrial DNA Control Region (CR) sequence data using existing amplification procedures and primers (Kirchman 2009, Ozaki et al. 2010), and augmented this with published CR sequences for buff-banded rail from Vanuatu and other Pacific islands (Kirchman and Franklin 2007, Kirchman 2009). Our analysis included a wide sampling of localities (48), subspecies (15) and archipelagos (12) (Fig. 2, Supplementary material Appendix 1, Table A2). We excluded three published sequences because the sequences on GenBank were different from the haplotypes reported in the related publication (EF219120 and EF219117) or the specific locality is unknown (EF219129). The new data represent additional individuals from localities in New Caledonia, North Island (New Zealand), Samoa, Australia and several islands in Fiji. Previously unsampled localities include Cocos Islands, Niue Island, South Island (New Zealand) and Sumba (Indonesia). Together these provide a graded spatial intra- and inter-archipelagic set with which to explore gene flow at different geographic scales. Control Region sequences are available under GenBank Accession numbers in Supplementary material Appendix 1, Table A1.

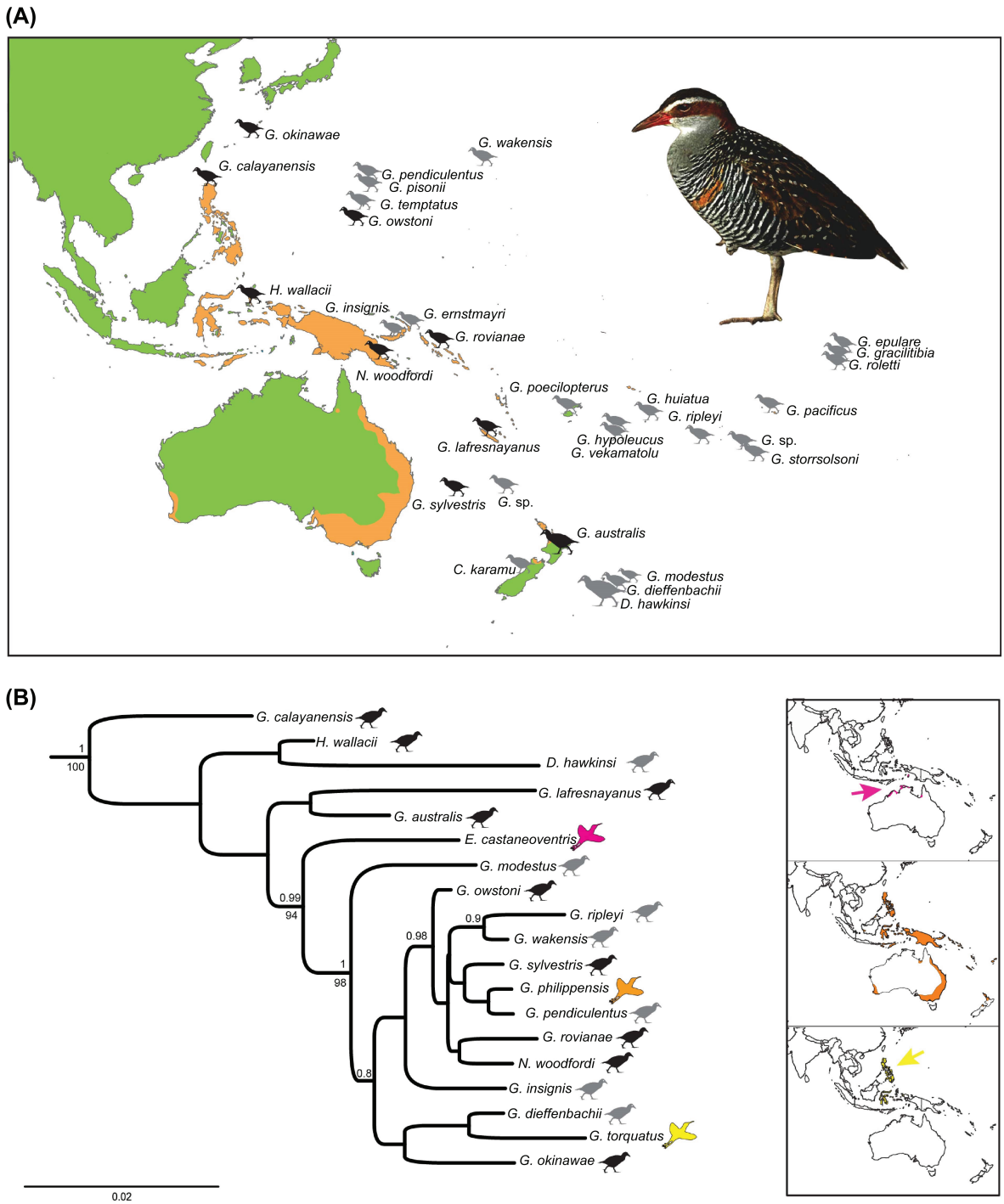


Figure 1. (A) Living (black) and extinct (gray) flightless insular species within *Gallirallus* in Western Pacific. Each flightless-bird icon shows a different endemic species in the location indicated. The sizes of the icons are proportional to the sizes reported for the birds. Orange shading indicates the geographical distribution of the volant *Gallirallus philippensis*. Several of the flightless species became extinct after human colonisation. (B) Bayesian phylogenetic tree from concatenated alignment sequences showing the *Gallirallus* group (modified from Fig. 1 of Garcia-R et al. 2016). Posterior probabilities over 0.8 and ML bootstrap supports over 70% are indicated above and below each node, respectively. Flighted species are shown with a winged-bird icon and flightless species with a walking-bird icon. Coloured winged-bird icons correspond to geographic range maps of volant species (right).

Traditionally, the history of colonisation events has been inferred from genetic data using 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 (Hey and Machado 2003). These model-based approaches have advantages over traditional population genetic analyses when studying gene flow and colonisation to understand the

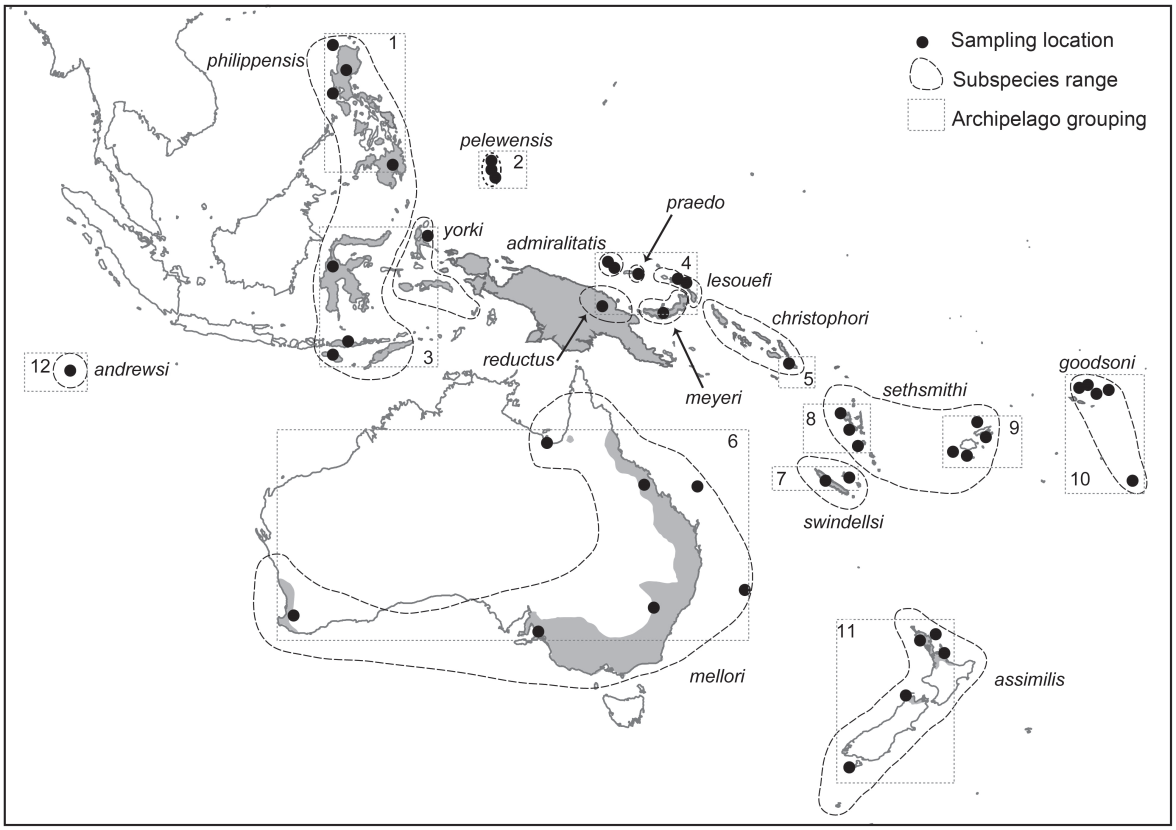


Figure 2. The Australasia region showing approximate sampling localities (small black circles) and sampled buff-banded rail *G. philippensis* subspecies (dotted lines) in 12 geographical groups (dashed boxes) where: 1 = Philippines, 2 = Palau, 3 = Wallacea, 4 = Bismarck, 5 = Solomon, 6 = Australia, 7 = New Caledonia, 8 = Vanuatu, 9 = Fiji, 10 = Samoa, 11 = New Zealand, 12 = Cocos Islands. Gray shading indicates the full geographical distribution of *G. philippensis*.

processes that occur during population divergence or speciation. Compared to descriptive measures of population differentiation, this method incorporates genetic stochasticity by sampling from the range of genealogies that are consistent with the data (Hey and Nielsen 2004).

We employed DnaSP ver. 5.0 to summarise genetic variation of each population, archipelago and subspecies (Librado and Rozas 2009) using: number of haplotypes (h), number of segregating sites (S), haplotype diversity (Hd), nucleotide diversity per site ($\pi$) and Watterson's estimator of the per-site population mutation rate ($\theta_W$). 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 Fu's *Fs* statistic 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, we calculated the $R_2$ statistic for populations, as 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*) indicate 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), a bottleneck (Fay and Wu 1999, Hammer et al. 2004), or low incidence of gene flow from other populations (Crawford 2003). Significantly positive values result from an excess of common variants relative to rare ones that 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 when applied to neutral markers.

Genetic divergence between each pair of population comparisons was quantified using Lynch and Crease's $F_{ST}$ (Lynch and Crease 1990) in DnaSP ver. 5.0 with 5000 replicates and a significance level of $\leq 0.05$. Haplotype networks were constructed in PopART ver. 1.7 (Leigh and Bryant 2015) using the minimum spanning algorithm to visualize the relationship between haplotypes and their geographical distribution. A Mantel test of the correlation between genetic (Wright's $F_{ST}$) and geographical distance (km) was conducted using the ade4 package (Dray and Dufour 2007) with 10 000 permutations implemented in the R programming environment (R Development Core Team). Correlation coefficients were also calculated to estimate the relationship between nucleotide diversity per site ($\pi$) and haplotype diversity (Hd) against land area of archipelagos ($km^2$). Archipelago areas, haplotype

diversity and nucleotide diversity per site were $\log_{10}$ transformed prior to the analyses.

We obtained estimates of effective population size ($N_e$), 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 buff-banded rail (Dunlop 1970, Garnett et al. 2011). We used the Hasegawa, Kishino and Yano (HKY) model of substitution (Hasegawa et al. 1985), due to unequal base frequencies, and several preliminary MCMC simulations with broad priors to establish appropriate bounds for each parameter. We 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).

To obtain $N_e$ from $\theta$, a mutation rate, $\mu$, of $4.7 \times 10^{-8}$ per site per year was applied to the CR data. Variation in mutation rate among mitochondrial genes has not been studied in these birds so to obtain this approximation we assumed the mutation rate was equal to the substitution rate for the mitochondrial Control Region (Ho et al. 2005). Although substitution rates vary dramatically among mitochondrial genes in animals and variation in rates of mutation is less dramatic, some analyses suggest that genes near origins of replication may have elevated mutation rates due to the slightly extended 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). We compared percent divergence in CR with percent divergence in the cytochrome *b* (cyt *b*) coding region sequences for two pairwise comparisons of buff-banded rail populations in Australia (Supplementary material Appendix 1, Table A1). Weir and 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*, we inferred that the CR mutates at a rate ($0.9\% \pm 0.3$) about 4.5 times faster than the cyt *b* ($0.2\% \pm 0.1$) coding region (range = 4.0 to 6.0 times faster). Thus, we estimated that the CR evolves at a rate of 9.5% sequence divergence per My or at a rate of $4.7 \times 10^{-8}$ substitutions per site per year ($3.0 \times 10^{-8}$ to $12 \times 10^{-8}$ substitutions per site per year).

### Microsatellite loci

Population genetic analysis was performed on microsatellite data using DNA from fresh samples of buff-banded rails collected in Australia, Cocos Islands, New Zealand, New Caledonia and Samoa. We examined DNA variation across six microsatellite loci using primers developed for *Crex crex* (Gautschi et al. 2002) and Australian *G. philippensis* (Manson 2003) (see Supplementary material Appendix 1, Table A3 for primer details). PCR reaction volumes of 10 µl contained 0.2 mM dNTPs, 1X buffer solution, 0.2 µM of reverse primer and 0.044 µM of forward primer with M13 tail, 2.5 mM $MgCl_2$, 0.5 U of Taq polymerase and 1–5 µl total DNA (Lux 2008). Forward primers were fluorescently labelled with either HEX, NED or FAM (Schuelke 2000). Thermal cycling involved one cycle of 94°C for 2 m; 40 cycles of 94° for 30 s, 50°C–60°C for 30 s, 72°C for 45 s, and a final extension of 72° for 10 min (Lux 2008). Amplified DNA was analyzed for length variation on an ABI 3730XL using the GeneScan-500 LIZ dye size standard and scored with the microsatellite plugin in Geneious ver. 6.0.5 (Drummond et al. 2012). The presence of null alleles, long-allele dropouts, and stuttering were tested using 1000 randomisations in MICRO-CHECKER ver. 2.2.3 (Van Oosterhout et al. 2004). The total number of alleles per locus and per population and the distribution of allele frequencies were determined using Genetic Data Analysis (GDA) (Lewis and Zaykin 2001). Deviations from Hardy–Weinberg equilibrium, expected ($H_E$) and observed ($H_O$) heterozygosity, and linkage disequilibrium were tested using Genepop ver. 4.1.4 (Rousset 2008), for each locus separately and over all loci for each sampling site with a Bonferroni correction to minimize type I errors (Rice 1989). To identify clusters of individual genotypes and estimate the optimal number of hypothetical populations (K) based on multi-locus microsatellite genotypes we used the programs STRUCTURE ver. 2.3.4 (Pritchard et al. 2000), STRUCTURE HARVESTER (Earl and vonHoldt 2012) and CLUMPP (Jakobsson and Rosenberg 2007). We performed 20 runs for every value of K using an admixture model with a burn-in of $1 \times 10^5$ Markov Chain Monte Carlo (MCMC) iterations and $1 \times 10^6$ follow-on MCMC iterations. We also estimated pairwise $F_{ST}$ (Weir and Cockerham 1984) between all pairs of populations using Arlequin ver. 3.5.1.2 (Excoffier et al. 2005) based on the results from STRUCTURE.

## Results

### Genetic diversity of mitochondrial CR gene

We analysed 433 bp of CR sequence data for 152 individuals of *G. philippensis* across Oceania and the Philippines. However, due to missing data in published sequences we used 290 bp of sequence for most analyses, since several software packages (e.g. DnaSP ver. 5.0 and PopART ver. 1.7) exclude gaps when performing analyses. We identified 49 haplotypes of which 38 had previously been reported (Kirchman 2009). Three haplotypes were widely distributed in the archipelagos of Wallacea, Bismarck, New Zealand, New Caledonia and Samoa (Hap 1); Cocos Islands, Wallacea, Australia and New Zealand (Hap 24); and Australia, New Zealand, Fiji and Samoa (Hap 40). A total of thirty-two endemic haplotypes were found in the 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) (Fig. 3A).

Nucleotide diversity ($\pi$) in sampling localities with $n \geq 2$ ranged from 0.0 (Niue, New Britain, New Caledonia, Heron Island) to 0.0153 (New Guinea) (Supplementary material Appendix 1, Table A4). At archipelago and subspecies level, the Cocos Islands ($\pi = 0.00249$) and *G. p. admiralitatis* and *G. p. meyeri* subspecies showed the lowest values ($\pi = 0.0$) contrasting with Palau and *G. p. philippensis* (0.0246 and

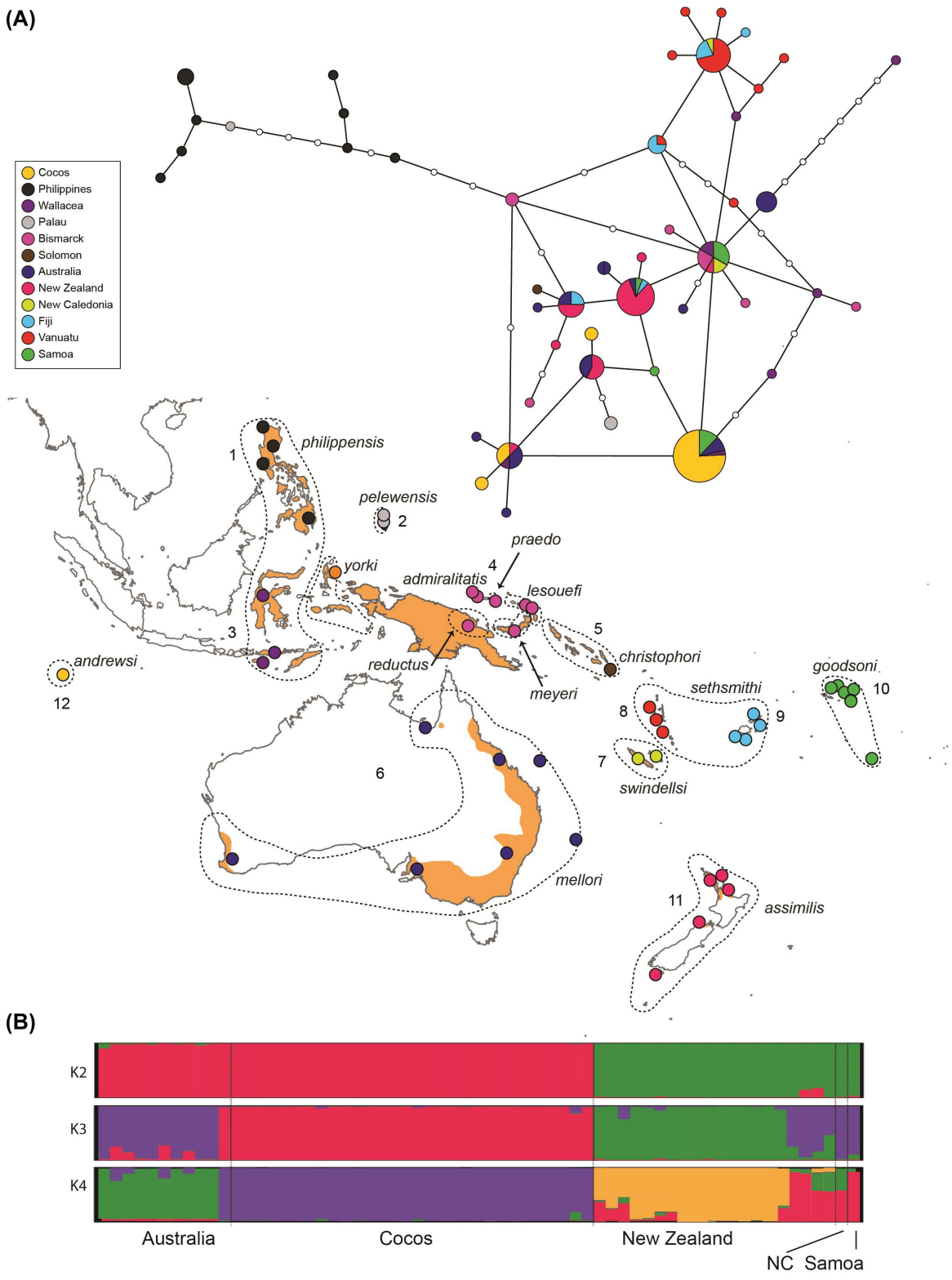


Figure 3. Distribution of genetic diversity in buff-banded rail *Gallirallus philippensis*. (A) Minimum spanning network of mtDNA Control Region haplotypes. Size of the circle is proportional to the frequency of individuals found for each haplotype. Nucleotide differences between haplotypes are indicated by one-step edges. Numbers of substitutions are indicated on each line connecting the haplotypes. Colours correspond to 12 major geographical regions representing archipelagos (Fig. 2). (B) Genotypic variation among populations of buff-banded rail in Oceania surveyed using six microsatellite loci and Bayesian assignment analysis in STRUCTURE (Pritchard et al. 2000).

0.0256, respectively; Table 1). Up to 9 segregating sites (S) were observed in each population, yielding a population mutation per site rate ($\theta_W$) from 0.0 to 0.0153 (Supplementary material Appendix 1, Table A4). The Philippines, Wallacea, Vanuatu and Palau and their respective subspecies presented the highest number of segregating 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 1). Nucleotide diversity declined in more remote archipelagos but haplotype diversity was not correlated with this and was similar for most archipelagos although Cocos Islands had the lowest value for both statistics (Fig. 4A). In neutrality tests none of the populations and archipelagos showed any significant skew in values of $D_T$, except Fu's *Fs* test in the NSW deme (Australia) and Australia, New Zealand and Vanuatu archipelagos (Table 1 and Supplementary material Appendix 1, Table A4). Fu's *Fs* results indicate departures from the Wright–Fisher neutral model for these populations. Subspecies showing significantly negative deviation from neutality according to 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 (Australia) ($R_2 = 0.137$, $p < 0.001$) and Santo (Vanuatu) ($R_2 = 0.112$, $p < 0.001$) at population level and Bismarck ($R_2 = 0.116$, $p < 0.05$) at archipelago level (Table 1 and Supplementary material Appendix 1, Table A4).

## Genetic structure and coalescent analyses

We 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 within New Zealand and within the Philippines (results not shown). These $F_{ST}$ results may reflect low sample size in some cases so we grouped samples 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 pairs of subspecies showed no evidence that New Caledonia (subspecies *G. p. swindellsi*) was distinct from the archipelagos of Vanuatu, Fiji, Samoa, Australia, Palau and Wallacea and the subspecies *G. p. sethsmithi* (Vanuatu and Fiji), *G. p. goodsoni* (Samoa), *G. p. mellori* (Australia), *G. p. pelewensis* (Palau) and *G. p. philippensis* (Philippines) (Table 2). 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 between Australia and New Zealand archipelagos and other archipelagos. The biggest values of highly significant divergence ($p < 0.001$) were mostly among Philippine samples. Estimates of divergence at subspecies level showed the smallest values of highly significant divergence ($p < 0.001$) in comparisons with *G. p. mellori,* and the biggest values of differentiation ($p < 0.001$) were mostly in comparisons with *G. p. andrewsi*.

Geographical association of haplotypes visualized with a network identified a similar structure; haplotypes from the Philippines archipelago were the most divergent from all others, and haplotypes found in Australia occurred widely in the haplotype network (Fig. 3A). There was a positive correlation between genetic difference ($F_{ST}$) and the spatial scale at archipelago level (Mantel test $r = 0.382$, $p = 0.012$; Fig. 4B). Furthermore, when we tested for a relationship between nucleotide diversity per site ($\pi$) and archipelago area ($km^2$), we found a positive correlation ($r = 0.75$, $p < 0.01$), but only when the Palau archipelago was excluded from the analysis ($r = 0.33$, $p > 0.05$ when Palau is included in the

Table 1. Variation among mtDNA Control Region sequences from populations of buff-banded rails *Gallirallus philippensis* grouped by archipelago (upper) and subspecies (lower). n = sample sizes, h = haplotype numbers, S = segregating sites, Hd = haplotype diversity, $\theta_W$ = population mutation rate per site, $\pi$ = nucleotide diversity per site, $D_T$ = Tajima's D test, Fs = Fu's *F*s test, $R_2$ = Ramos and Rozas statistic. NA = not applicable. * = $0.01 < p < 0.05$; ** = $0.001 < p < 0.01$; *** = $< 0.001$. Genetic polymorphism information for individual localities with more than two samples is in Supplementary material Appendix 1, Table A4.

| | n | h | S | Hd | $\theta_W$ | $\pi$ | $D_T$ | *F*s | $R_2$ |
|---|---|---|---|---|---|---|---|---|---|
| Archipelagos | | | | | | | | | |
| Cocos | 30 | 3 | 4 | 0.301 | 0.003 | 0.0025 | –0.048 | 1.590 | 0.123 |
| Philippines | 10 | 8 | 12 | 0.933 | 0.013 | 0.0143 | 0.428 | –2.190 | 0.169 |
| Wallacea | 8 | 7 | 12 | 0.964 | 0.016 | 0.0126 | –1.032 | –2.671 | 0.215 |
| Australia | 22 | 11 | 10 | 0.913 | 0.009 | 0.0090 | –0.165 | **–4.126*** | 0.119 |
| New Zealand | 27 | 8 | 7 | 0.707 | 0.005 | 0.0040 | –0.853 | **–3.080*** | 0.089 |
| Fiji | 10 | 5 | 4 | 0.844 | 0.003 | 0.0039 | 0.806 | –0.962 | 0.192 |
| Vanuatu | 19 | 9 | 12 | 0.731 | 0.008 | 0.0051 | –1.271 | **–3.032*** | 0.090 |
| Samoa | 10 | 4 | 2 | 0.733 | 0.002 | 0.0028 | 0.931 | –1.074 | 0.227 |
| Bismarck | 9 | 6 | 8 | 0.889 | 0.009 | 0.00684 | –1.112 | –1.901 | **0.116*** |
| Palau | 3 | 2 | 12 | 0.667 | 0.025 | 0.0246 | NA | NA | 0.471 |
| New Caledonia | 3 | 2 | 2 | 0.667 | 0.004 | 0.0041 | NA | NA | 0.471 |
| Subspecies | | | | | | | | | |
| *G. p. andrewsi* | 30 | 3 | 4 | 0.301 | 0.002 | 0.002 | –0.048 | 1.590 | 0.123 |
| *G. p. philippensis* | 17 | 14 | 18 | 0.971 | 0.018 | 0.026 | 1.548 | **–4.590**** | 0.197 |
| *G. p. pelewensis* | 3 | 2 | 12 | 0.667 | 0.025 | 0.025 | NA | NA | 0.471 |
| *G. p. reductus* | 2 | 2 | 5 | 1.000 | 0.015 | 0.015 | NA | NA | 0.500 |
| *G. p. admiralitatis* | 2 | 1 | 0 | 0.000 | 0.000 | 0.000 | NA | NA | 0.500 |
| *G. p. lesouefi* | 2 | 2 | 1 | 1.000 | 0.003 | 0.003 | NA | NA | 0.500 |
| *G. p. meyeri* | 2 | 1 | 0 | 0.000 | 0.000 | 0.000 | NA | NA | NA |
| *G. p. mellori* | 22 | 11 | 10 | 0.913 | 0.009 | 0.009 | –0.165 | **–4.126*** | 0.119 |
| *G. p. assimilis* | 27 | 8 | 7 | 0.707 | 0.005 | 0.004 | –0.853 | **–3.080*** | 0.089 |
| *G. p. swindellsi* | 3 | 2 | 2 | 0.667 | 0.004 | 0.004 | NA | NA | 0.471 |
| *G. p. sethsmithi* | 29 | 12 | 13 | 0.788 | 0.008 | 0.005 | –1.207 | **–5.328**** | 0.078 |
| *G. p. goodsoni* | 10 | 4 | 2 | 0.733 | 0.002 | 0.003 | –0.931 | –1.074 | 0.227 |

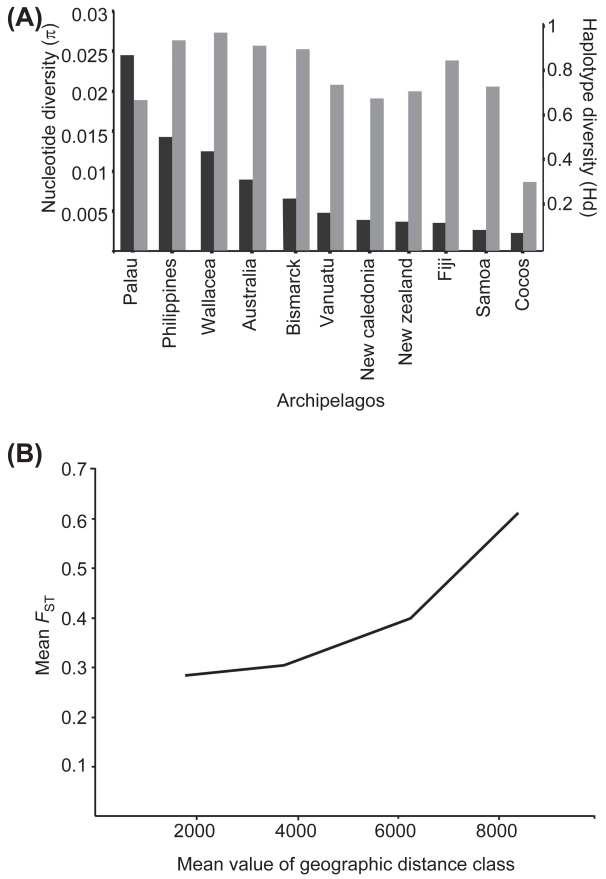


Figure 4. Genetic diversity plot of buff-banded rail. (A) Nucleotide diversity (black bars) and haplotype diversity (gray bars) among archipelagos. (B) Distogram showing the relationship between mean pairwise $F_{ST}$ within distance classes.

correlation). There was also a positive correlation between haplotype diversity (Hd) and archipelago area (km$^2$), without exclusion of the Palau archipelago data ($r = 0.83$, $p < 0.01$).

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 Islands, 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 (Fig. 5, Supplementary material Appendix 1, Table A5) and only one inferred case of back-gene flow from Vanuatu to Australia and New Zealand.

## Population genetics analyses of microsatellite data

The probability of null alleles, long-allele dropout, and stuttering were negligible for all loci. The number of alleles at the six microsatellite loci surveyed in buff-banded rails ranged from two to 11 and observed heterozygosity was moderate to high in the Cocos Islands and Australian samples, but low in New Zealand (with most loci $< 0.2$) (Supplementary material Appendix 1, Table A6). All loci deviated from Hardy–Weinberg equilibrium in New Zealand ($p < 0.001$), but not in Cocos Islands and Australian populations. The inbreeding coefficient ($F_{IS}$) ranged from –0.27 to 0.93 and the exact G-test was significant ($p < 0.001$) showing that the sampled alleles were not drawn from the same distribution in all populations. STRUCTURE analysis identified three genetic demes ($K = 3$) as the most probable number of genetic clusters within our dataset (Fig. 3B). These genetic clusters correspond to the Australian, Cocos Islands and New Zealand populations with samples from New Caledonia ($n = 1$) and Samoa ($n = 1$) attributable to the Australian group. At $K = 3$ the Samoan, New Caledonian and some New Zealand (GBI and Northland) samples were grouped with Australian samples but one Australian sample from NSW clustered with the Cocos Islands sample. However, at $K = 4$ these specimens form a separate cluster (Fig. 3B) with exception of the single Australian sample that clustered with Cocos Islands. Despite a relative high number of migrants (0.31), pairwise $F_{ST}$ values yielded moderate to high genetic differentiation

Table 2. Population genetic variation among populations of buff-banded rail *Gallirallus philippensis*. $F_{ST}$ values between 11 (Solomon excluded) major geographical regions (1–11 above the diagonal) and 12 subspecies (A–L below the diagonal). Regular font = $0.01 < p < 0.05$; * = $0.001 < p < 0.01$; bold font $p < 0.001$. *Gallirallus reductus* (A), *admiralitatis* (B), *lesouefi* (C), *meyeri* (D), *swindellsi* (E), *sethsmithi* (F), *goodsoni* (G), *philippensis* (H), *pelewensis* (I), *assimilis* (J), *mellori* (K), *andrewsi* (L). Bismarck (1), New Caledonia (2), Vanuatu (3), Fiji (4), Samoa (5), Wallacea (6), Philippines (7), Palau (8), New Zealand (9), Australia (10), Cocos (11).

| | A | B | C | D | E | F | | G | H | | I | J | K | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subspecies | 1 | | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Archipelagos |
| A | | | | | | | | | | | | | | | |
| B | 0.285 | | | | 0.000 | **0.328** | 0.230* | 0.184 | 0.043 | **0.668** | 0.323* | **0.321** | 0.144* | **0.446** | 1 |
| C | 0.000 | 0.800 | | | | | | | | | | | | | |
| D | 0.000 | 0.900 | 0.000 | | | | | | | | | | | | |
| E | –0.050 | 0.714 | 0.000 | 0.000 | | 0.131 | 0.028 | 0.178 | 0.029 | **0.718** | 0.382 | 0.399 | 0.206 | 0.539* | 2 |
| F | 0.137 | 0.660 | 0.425 | 0.522 | 0.087 | | 0.055 | **0.476** | **0.284** | **0.697** | **0.393** | **0.498** | **0.380** | **0.685** | 3 |
| | | | | | | | | **0.424** | **0.246** | **0.693** | 0.372* | **0.404** | **0.312** | **0.706** | 4 |
| G | 0.076 | 0.831 | 0.203 | 0.349 | 0.178 | **0.452** | | | 0.012 | **0.738** | 0.328* | **0.362** | 0.082* | 0.289* | 5 |
| H | 0.243 | 0.387 | 0.400 | 0.431 | 0.370 | **0.384** | | 0.390* | | **0.638** | 0.266* | **0.263** | 0.035 | **0.154** | 6 |
| | | | | | | | | | | | 0.278 | **0.685** | **0.653** | **0.771** | 7 |
| I | 0.290 | 0.368 | 0.400 | 0.428 | 0.382 | **0.383** | | 0.328* | 0.049 | | | **0.224** | **0.195** | **0.354** | 8 |
| J | 0.185* | **0.771** | 0.449 | 0.590 | 0.399 | **0.464** | | **0.362** | **0.339** | | **0.224** | | **0.187** | **0.640** | 9 |
| K | 0.090* | 0.544 | 0.236 | 0.299 | 0.206 | **0.352** | | 0.082 | **0.299** | | **0.195** | **0.187** | | **0.234** | 10 |
| L | 0.274* | **0.928** | 0.591* | 0.831 | 0.539* | **0.686** | | 0.289* | **0.448** | | **0.354** | **0.641** | **0.234** | | 11 |

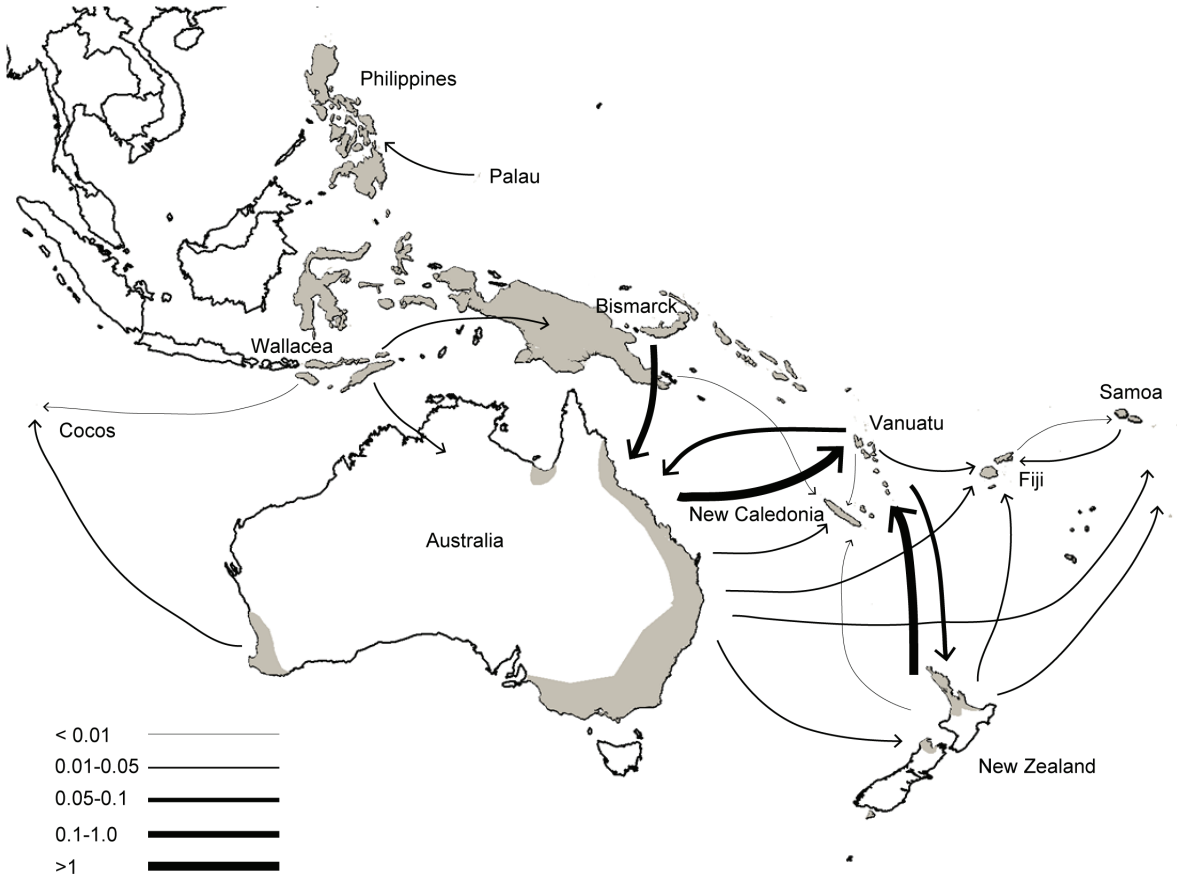


Figure 5. The likely directions of historic dispersal and colonisation events of buff-banded rail in Oceania inferred from simulations in IMa (Hey and Nielsen 2007). Arrows indicate direction of dispersion and their relative thickness is scaled to the extent of gene flow between sampled archipelagos.

among all populations with exception of one locus (Locus 11) that showed low differentiation (0.08).

## Discussion

Phylogenetic analysis of extant and recently extinct insular *Gallirallus* reveals a shallow and potentially polytomous pattern consistent with contemporaneous radiation (Fig. 1B). Available evidence indicates that this radiation involved independent evolution of island endemics from volant colonist populations in this lineage (Trewick 1997a, Garcia-R et al. 2014). Here we inferred genetic structure for a volant species that simulates the ecotype of the ancestral colonist for many flightless island endemics in the same lineage. The volant species, *G. philippensis*, has a range spanning many islands and archipelagos and this brings it into sympatry with island endemics with which it is related. This raises the question of how intraspecific dispersal rates intersect with divergence and speciation. The coexistence of a vagile 'tramp' and insular endemics indicates drift associated with founding events, intense local selection that overcomes gene flow among populations or increase in speciation rates after islands are colonised. High speciation rates have been found even in small islands where ecological divergence is less likely and extinction can increase (Schluter 1998). Alternatively, perhaps dispersal rates among populations of the volant species are not so high as the wide spatial range suggests. The recognition of more than 20 subspecies within the buff-banded rail (Schodde and Naurois 1982, Marchant and Higgins 1993), however, could be explained equally by regional selection or genetic drift after range expansion. Both selection and drift would be facilitated by low gene flow among regions. We therefore tested the null hypothesis of a panmictic population and even pattern of gene flow among buff-banded rail populations. More explicitly, we expect populations to be genetically more similar in the presence of a constant rate of gene flow correlated with physical attributes such as geographic distance and neighbouring populations.

Analysis of mtDNA haplotype data revealed a generalised pattern of reduced genetic diversity in the more remote archipelagos. We found that genetic diversity tended to decrease and genetic differentiation ($F_{ST}$) increase as the geographic distance separating populations increased (Fig. 4). Fine-scale analysis of microsatellite genotype data from selected populations revealed a signal for spatial partitioning that coincides with landscape connectivity and subspecies classification, with genetic similarity linked to proximity. Thus, in the west of the range, Australian and Cocos Islands individuals tended to have similar genotypes, while eastwards, New Zealand, New Caledonia and Samoa share similarity. We note, however, that some individuals in New Zealand appear to have genotypes more similar to those in New Caledonia and Samoa than others in New Zealand. This could reflect

mixing between those places, or partitioning of the disjunct northern and southern populations in New Zealand.

The isolation-with-migration model-based method (IMa) offers options for a simplified set of assumptions about population history (e.g. strict neutrality and no recombination) and imposes some limitations (e.g. mutation models and resolution from a single locus) in order to infer demographic parameters (Hey and Nielsen 2004, Pinho and Hey 2010). Demographic parameter estimates generated by IMa are however generally robust to violations of these conditions (Strasburg and Rieseberg 2010) and our findings are likely consistent with this view. Coalescent analysis of haplotype data indicates variation in levels of gene flow among archipelagos (Fig. 5, Supplementary material Appendix 1, Table A5). The large 90% HPD intervals, along with significant negative Fu's *Fs* values and significant positive $R_2$ values, suggest that the buff-banded rail has recently expanded its range in Australia, Vanuatu, New Zealand and Bismarck. This leads to uncertainty in gene flow estimates because recent range expansions will mimic high rates of gene flow between distant populations (Slatkin 1993). These results then are less comprehensible for 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, estimates of a high level of gene flow, population sizes and similar divergence times among populations indicate that the key to formation of insular populations within this lineage might be strong selection pressures and not the loss of rare alleles by genetic drift during range expansion. Haplotype sharing in the mtDNA network and Bayesian assignment analysis of microsatellites data could also be due to some ongoing gene flow between regions/subspecies with the exception of Philippines and Palau that contain unique endemic haplotypes. However, the signal of population differentiation across localities and subspecies indicates the contrary, i.e. restriction of gene flow. The recognition of morphologically differentiated subspecies in buff-banded rail (Schodde and Naurois 1982, Marchant and Higgins 1993) is an indication that divergent natural selection is not countered by the homogenizing effects of gene flow.

Our population genetic results are consistent with subspecies taxonomy and geographic space (archipelagos). Further, they support a model of colonisation from the biggest islands to the nearest neighbouring islands, and onwards to more remote islands. Buff-banded rails show a pattern of colonisation where Australia has been a net source of dispersal for many islands in Oceania. Neutral genetic differentiation suggests dispersal events involving numerous individuals as Australia shares haplotypes with five different archipelagos including those otherwise distantly separated in Wallacea and Samoa. Isolated populations in the Samoan and Cocos archipelagos have lower genetic variation than populations closer to Australia, which appear to have experienced admixture from multiple sources (Schodde and Naurois 1982).

The minimum linear over-sea distances between the most isolated archipelagos (Samoa and Cocos Islands) are about 10 000 km, and yet the minimum number of non-sister haplotypes indicates colonisation and establishment by groups of individuals. The most parsimonious explanation is that dispersal of buff-banded rail is episodic. The co-occurrence with insular flightless endemics is likely the result of separate waves of dispersal followed by periods of low gene flow between conspecific populations. The phylogenetic polytomy among *Gallirallus* (Fig. 1B) is consistent with a model of periodic expansion between islands and rapid speciation of a phenotypically plastic ancestor. Directional asymmetry of dispersal (Cook and Crisp 2005) involving flying *Gallirallus* is strongly influenced by a tendency toward flightlessness. The evolution of flightlessnes yields energy conservation where ground predators are few (McNab 2002) and substantially reduces the likelihood of further dispersal.

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## References

Beauchamp, A. J. 1989. Panbiogeography and rails of the genus *Gallirallus*. – N. Z. J. Zool. 16: 763–772.

Bellemain, E. and Ricklefs, R. E. 2008. Are islands the end of the colonization road? – Trends Ecol. Evol. 23: 461–468.

Braverman, J. M., Hudson, R. R., Kaplan, N. L., Langley, C. H. and Stephan, W. 1995. The hitchhiking effect on the site frequency spectrum of DNA polymorphisms. – Genetics 140: 783–96.

Broughton, R. E. and Reneau, P. C. 2006. Spatial covariation of mutation and nonsynonymous substitution rates in vertebrate mitochondrial genomes. – Mol. Biol. Evol. 23: 1516–1524.

Brown, W. M., George, M. and Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. – Proc. Natl Acad. Sci. USA 76: 1967–1971.

Clegg, S. 2010. Evolutionary changes following island colonization in birds: empirical insights into the roles of microevolutionary processes. – In: Losos, J. and Ricklefs, R. (eds), The theory of island biogeography revisited. Princeton Univ. Press, pp. 293–324.

Clegg, S. M., Degnan, S. M., Kikkawa, J., Moritz, C., Estoup, A. and Owens, I. P. F. 2002. Genetic consequences of sequential founder events by an island-colonizing bird. – Proc. Natl Acad. Sci. USA 99: 8127–8132.

Cook, L. G. and Crisp, M. D. 2005. Directional asymmetry of long-distance dispersal and colonization could mislead reconstructions of biogeography. – J. Biogeogr. 32: 741–754.

Crawford, A. J. 2003. Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. – Mol. Ecol. 12: 2525–2540.

Diamond, J. 1975. Assembly of species communities. – In: Cody, M. and Diamond, J. (eds), Ecology and evolution of communities. Harvard Univ. Press, pp. 342–444.

Diamond, J. 1977. Continental and insular speciation in Pacific land birds. – Syst. Biol. 26: 263–268.

Diamond, J. 1991. A new species of rail from the Solomon islands and convergent evolution of insular flightlessness. – Auk 108: 461–470.

Dray, S. and Dufour, A. B. 2007. The ade4 package: implementing the duality diagram for ecologists. – J. Stat. Softw. 22: 1–20.
Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. and Wilson, A. 2012. Geneious v.6.05. – <www.geneious.com>.
Dunlop, R. R. 1970. Behaviour of the banded rail, *Rallus philippensis*. – Sunbird 1: 3–15.
Earl, D. and vonHoldt, B. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – Conserv. Genet. Resour. 4: 359–361.
Edelaar, P. and Bolnick, D. I. 2012. Non-random gene flow: an underappreciated force in evolution and ecology. – Trends Ecol. Evol. 27: 659–665.
Edelaar, P., Siepielski, A. M. and Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. – Evolution 62: 2462–2472.
Elliott, G. 1987. Habitat use by the banded rail. – N. Z. J. Ecol. 10: 109–115.
Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. – Evol. Bioinform. Online 1: 47–50.
Fay, J. C. and Wu, C.-I. 1999. A human population bottleneck can account for the discordance between patterns of mitochondrial versus nuclear DNA variation. – Mol. Biol. Evol. 16: 1003–1005.
Filardi, C. E. and Moyle, R. G. 2005. Single origin of a pan-Pacific bird group and upstream colonization of Australasia. – Nature 438: 216–219.
Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. – Genetics 147: 915–925.
Garcia-R, J. C., Gibb, G. C. and Trewick, S. A. 2014. Deep global evolutionary radiation in birds: diversification and trait evolution in the cosmopolitan bird family Rallidae. – Mol. Phylogenet. Evol. 81: 96–108.
Garcia-R, J. C., Elliott, G., Walker, K., Castro, I. and Trewick, S. A. 2016. Trans-equatorial range of a land bird lineage (Aves: Rallidae) from tropical forests to subantarctic grasslands. – J. Avian Biol. 47: 219–226.
Garnett, S., Szabo, J. and Dutson, G. 2011. Subspecies conservation summary: buff-banded rail Cocos Islands. The action plan for Australian birds. – CSIRO Publishing.
Gautschi, B., Arter, M. K., Husi, R. E., Wettstein, W. and Schmid, B. 2002. Isolation and characterization of microsatellite loci in the globally endangered corncrake, *Crex crex* Linne. – Conserv. Genet. 3: 451–453.
Goldberg, J., Trewick, S. A. and Powlesland, R. G. 2011. Population structure and biogeography of *Hemiphaga* pigeons (Aves: Columbidae) on islands in the New Zealand region. – J. Biogeogr. 38: 285–298.
Grant, P. R., Grant, B. R. and Petren, K. 2001. A population founded by a single pair of individuals: establishment, expansion, and evolution. – Genetica 112–113: 359–382.
Hammer, M. F., Garrigan, D., Wood, E., Wilder, J. A., Mobasher, Z., Bigham, A., Krenz, J. G. and Nachman, M. W. 2004. Heterogeneous patterns of variation among multiple Human X-Linked loci: the possible role of diversity-reducing selection in non-Africans. – Genetics 167: 1841–1853.
Hasegawa, M., Kishino, H. and Yano, T.-A. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. – J. Mol. Evol. 22: 160–174.
Hey, J. 2010. The divergence of chimpanzee species and subspecies as revealed in multipopulation isolation-with-migration analyses. – Mol. Biol. Evol. 27: 921–933.
Hey, J. and Machado, C. A. 2003. The study of structured populations – new hope for a difficult and divided science. – Nat. Rev. Genet. 4: 535–543.
Hey, J. and Nielsen, R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. – Genetics 167: 747–760.
Hey, J. and Nielsen, R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. – Proc. Natl Acad. Sci. USA 104: 2785–2790.
Ho, S. Y. W., Phillips, M. J., Cooper, A. and Drummond, A. J. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. – Mol. Biol. Evol. 22: 1561–1568.
Ingman, M., Kaessmann, H., Paabo, S. and Gyllensten, U. 2000. Mitochondrial genome variation and the origin of modern humans. – Science 408: 708–713.
Jakobsson, M. and Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics 23: 1801–1806.
Jonsson, K. A. and Holt, B. G. 2015. Islands contribute disproportionately high amounts of evolutionary diversity in passerine birds. – Nat. Comm. 6.
Kirchman, J. J. 2009. Genetic tests of rapid parallel speciation of flightless birds from an extant volant ancestor. – Biol. J. Linn. Soc. 96: 601–616.
Kirchman, J. J. and Franklin, J. D. 2007. Comparative phylogeography and genetic structure of Vanuatu birds: control region variation in a rail, a dove, and a passerine. – Mol. Phylogenet. Evol. 43: 14–23.
Kolbe, J. J., Glor, R. E., Rodriguez Schettino, L., Lara, A. C., Larson, A. and Losos, J. B. 2004. Genetic variation increases during biological invasion by a Cuban lizard. – Nature 431: 177–181.
Leigh, J. W. and Bryant, D. 2015. popart: full-feature software for haplotype network construction. – Methods Ecol. Evol. 6: 1110–1116.
Lewis, P. O. and Zaykin, D. 2001. Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). – Free program distributed by the authors over the internet from <http://lewis.eeb.uconn.edu/lewishome/software.html>.
Librado, P. and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. – Bioinformatics 25: 1451–1452.
Lux, F. 2008. The conservation genetics of the cocos buff-banded rail *Gallirallus philippensis andrewsi*. – Botany and Zoology School, Australian National Univ., Australia.
Lynch, M. and Crease, T. J. 1990. The analysis of population survey data on DNA sequence variation. – Mol. Biol. Evol. 7: 377–394.
MacArthur, R. H. and Wilson, E. O. 1967. The theory of island biogeography. – Princeton Univ. Press.
Manson, F. J. 2003. Life history traits, social mating system and genetic variation in the buff-banded rail *Gallirallus philippensis* on Heron Island. – Dept of Zoology and Entomology, Univ. of Queensland, Brisbane.
Marchant, S. and Higgins, P. J. 1993. Handbook of Australian, New Zealand & Antarctic birds. – Oxford Univ. Press.
Maynard Smith, J. and Haigh, J. 1974. The hitch-hiking effect of a favourable gene. – Genet. Res. 23: 23–35.
Mayr, E. and Diamond, J. 2001. The birds of northern Melanesia: speciation, ecology and biogeography. – Oxford Univ. Press.
McNab, B. K. 2002. Minimizing energy expenditure facilitates vertebrate persistence on oceanic islands. – Ecol. Lett. 5: 693–704.

Moritz, C., Dowling, T. E. and Brown, W. M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. – Annu. Rev. Ecol. Syst. 18: 269–292.
Moyle, R. G., Filardi, C. E., Smith, C. E. and Diamond, J. 2009. Explosive Pleistocene diversification and hemispheric expansion of a "great speciator". – Proc. Natl Acad. Sci. USA 106: 1863–1868.
Neall, V. E. and Trewick, S. A. 2008. The age and origin of the Pacific islands: a geological overview. – Phil. Trans. R. Soc. B 363: 3293–3308.
Ozaki, K., Yamamoto, Y. and Yamagishi, S. 2010. Genetic diversity and phylogeny of the endangered Okinawa rail, *Gallirallus okinawae*. – Genes Genet. Syst. 85: 55–63.
Petit, R. J. and Excoffier, L. 2009. Gene flow and species delimitation. – Trends Ecol. Evol. 24: 386–393.
Pinho, C. and Hey, J. 2010. Divergence with gene flow: models and data. – Annu. Rev. Ecol. Evol. Syst. 41: 215–230.
Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inferences of population structure using multilocus genotype data. – Genetics 155: 945.
Ramirez-Soriano, A., Ramos-Onsins, S. E., Rozas, J., Calafell, F. and Navarro, A. 2008. Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. – Genetics 179: 555–567.
Ramos-Onsins, S. E. and Rozas, J. 2002. Statistical properties of new neutrality tests against population growth. – Mol. Biol. Evol. 19: 2092–2100.
Rice, W. R. 1989. Analyzing tables of statistical tests. – Evolution 43: 223–225.
Ripley, S. D. 1977. Rails of the world: a monograph of the Family Rallidae. – David R. Godine Publisher.
Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. – Mol. Ecol. Resour. 8: 103–106.
Schluter, D. 1998. Ecological causes of speciation. – In: Howard, D. J. and Berlocher, S. H. (eds), Endless forms: species and speciation. Oxford Univ. Press, p. 470.
Schodde, R. and Naurois, R. D. 1982. Patterns of variation and dispersal in the buff-breasted rail (*Gallirallus philippensis*) in the South-west Pacific, with description of a new subspecies. – Notornis 29: 131–141.
Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. – Nat. Biotech. 18: 233–234.
Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. – Evolution 47: 264–279.
Stockman, A. K. and Bond, J. E. 2007. Delimiting cohesion species: extreme population structuring and the role of ecological interchangeability. – Mol. Ecol. 16: 3374–3392.
Strasburg, J. L. and Rieseberg, L. H. 2010. How robust are "isolation with migration" analyses to violations of the IM model? A simulation study. – Mol. Biol. Evol. 27: 297–310.
Tajima, F. 1989a. The effect of change in population size on DNA polymorphism. – Genetics 123: 597–601.
Tajima, F. 1989b. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. – Genetics 123: 585–595.
Taylor, B. 1998. Rails: a guide to the rails, crakes, gallinules and coots of the world. – Yale Univ. Press.
Trewick, S. 2011. Vicars and vagrants. – Australasian Sci. 32: 22–25.
Trewick, S. A. 1997a. Flightlessness and phylogeny amongst endemic rails (Aves: Rallidae) of the New Zealand region. – Phil. Trans. R. Soc. B 352: 429–446.
Trewick, S. A. 1997b. Sympatric flightless rails *Gallirallus dieffenbachii* and *G. modestus* on the Chatham Islands, New Zealand; morphometrics and alternative evolutionary scenarios. – J. R. Soc. N. Z. 27: 451–464.
Trewick, S. A. and Gibb, G. C. 2010. Vicars, tramps and assembly of the New Zealand avifauna: a review of molecular phylogenetic evidence. – Ibis 152: 226–253.
Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. micro-checker: software for identifying and correcting genotyping errors in microsatellite data. – Mol. Ecol. Notes 4: 535–538.
Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. – Phil. Trans. R. Soc. B 367: 451–460.
Wallace, A. 1876. The geographical distribution of animals, with a study of the relations of living and extinct faunas as elucidating the past changes of the earth's surface. – Macmillan.
Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. – Evolution 38: 1358–1370.
Weir, J. T. and Schluter, D. 2008. Calibrating the avian molecular clock. – Mol. Ecol. 17: 2321–2328.
Young, A., Boyle, T. and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. – Trends Ecol. Evol. 11: 413–418.

Supplementary material (Appendix JAV-01201 at <www.avianbiology.org/appendix/jav-01201>). Appendix 1.