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The influence of gene flow and drift on genetic and phenotypic divergence in two species of *Zosterops* in Vanuatu

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Colonization of an archipelago sets the stage for adaptive radiation. However, some archipelagos are home to spectacular radiations, while others have much lower levels of diversification. The amount of gene flow among allopatric populations is one factor proposed to contribute to this variation. In island colonizing birds, selection for reduced dispersal ability is predicted to produce changing patterns of regional population genetic structure as gene flow-dominated systems give way to drift-mediated divergence. If this transition is important in facilitating phenotypic divergence, levels of genetic and phenotypic divergence should be associated. We consider population genetic structure and phenotypic divergence among two co-distributed, congeneric (Genus: *Zosterops*) bird species inhabiting the Vanuatu archipelago. The more recent colonist, *Z. lateralis*, exhibits genetic patterns consistent with a strong influence of distance-mediated gene flow. However, complex patterns of asymmetrical gene flow indicate variation in dispersal ability or inclination among populations. The endemic species, *Z. flavifrons*, shows only a partial transition towards a drift-mediated system, despite a long evolutionary history on the archipelago. We find no strong evidence that gene flow constrains phenotypic divergence in either species, suggesting that levels of inter-island gene flow do not explain the absence of a radiation across this archipelago.

Keywords: Vanuatu; regional population structure; gene flow; allopatric divergence; island birds

1. INTRODUCTION

The role of geographical separation and consequent elimination of gene flow in driving population divergence was a central tenet of the speciation models that emerged from the modern synthesis (Dobzhansky 1937; Mayr 1942, 1954). While allopatric speciation, with zero or very low levels of gene flow, is probably the most common geographic mode for many taxa (Barraclough & Vogler 2000; Coyne & Orr 2004; Phillimore *et al.* 2008*a*; Price 2008), some contemporary views place greater emphasis on ecological divergence and diversifying selection pressures, rather than the geographic context of divergence (Ogden & Thorpe 2002; Jordan *et al.* 2005; Rundle & Nosil 2005; Bolnick & Fitzpatrick 2007; Butlin *et al.* 2008; Nosil 2008). Nonetheless, in birds, only a few cases of potential sympatric or parapatric speciation have been identified (Sorenson *et al.* 2003; Huber *et al.* 2007; Ryan *et al.* 2007; Smith & Friesen 2007) and the majority of sister-species have contemporary range distributions that do not overlap (Barraclough & Vogler 2000; Coyne & Price 2000; Phillimore *et al.* 2008*a*).

Fragmented landscapes, such as archipelagos, provide a tractable setting to assess the influence of geography on genetic and phenotypic divergence (e.g. Clegg *et al.* 2002*a*; Petren *et al.* 2005; Jordan & Snell 2008; Phillimore *et al.* 2008*b*). For insular populations at the beginning of the speciation continuum (*sensu* Mayr & Diamond (2001), e.g. populations lacking (subspecific) geographic variation, subspecies or allospecies), the characterization of patterns of genetic connectivity among island populations can contribute to an understanding of the influence of geographical isolation on gene flow patterns and phenotypic divergence.

Correspondence between geographic and genetic isolation largely depends on an organism's vagility (Burney & Brumfield 2009), an attribute that can change over time in island colonizing species. Evidence from plants (Cody & Overton 1996) and birds (McNab 1994; Adler *et al.* 1995) suggests that island dwelling can result in selection for reduced dispersal abilities (see also Grant 1998). The changing patterns of population connectivity among islands are expected to produce distinct signatures of regional population genetic structure at neutral loci (Hutchison & Templeton 1999). If the colonizing ability of a species were maintained over time, then there would be weak

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relationships expected between the level of geographic isolation and genetic variation within and among populations.

Even in highly vagile organisms, ongoing within-archipelago dispersal and gene flow can be affected by behavioural or morphological changes that reduce the inclination or ability to effectively disperse across water barriers (Diamond *et al.* 1976; Mayr & Diamond 2001). Distance-limited dispersal is expected to generate a gene flow-mediated population structure evidenced by a negative relationship between neutral genetic diversity and geographic isolation (Jordan & Snell 2008), the establishment of isolation-by-distance patterns of genetic differentiation (Slatkin 1993; Hutchison & Templeton 1999) and low probabilities of alleles being identical by descent within populations (Ciofi *et al.* 1999). If geographically separated populations become progressively more genetically isolated over time, gene flow is expected to yield to drift as the predominant mechanism shaping population structure, producing a positive relationship between neutral genetic diversity and population size (approximated by island area), but not isolation (Lande & Barrowclough 1987; e.g. Hinten *et al.* 2003; Jordan & Snell 2008), a breakdown of isolation by distance patterns (Hutchison & Templeton 1999) and a high probability that alleles within a population are identical by descent (Ciofi *et al.* 1999). These represent extremes in a spectrum and intermediate patterns may also arise as regional population structure shifts, e.g. partial isolation-by-distance patterns (Hutchison & Templeton 1999) and generation of geographic patterns for types of variation most sensitive to demographic change (e.g. allelic diversity rather than heterozygosity; Nei *et al.* 1975).

The importance of gene flow as a process retarding phenotypic divergence is expected to vary considerably, depending on whether new mutations are universally favoured (Price 2008; Rundell & Price 2009; Schluter 2009) and on the strength of divergent selection (Lenormand 2002; Rundle & Nosil 2005). If phenotypic divergence is influenced by new mutations that are favoured in all populations, then a trickle of gene flow may be sufficient to prevent phenotypic divergence (Price 2008; Rundell & Price 2009). If divergent selection pressures exist, but are overwhelmed by gene flow, then this will also prevent phenotypic divergence. Under these two scenarios we would predict greater phenotypic divergence among drift-mediated, rather than among migration-mediation populations (Lenormand 2002). It is only if divergent selection is strong relative to levels of gene flow that we would predict substantial phenotypic divergence regardless of whether a populations genetic structure is drift- or migration-mediated (e.g. divergence with gene flow scenarios; Jordan *et al.* 2005; Nosil 2008).

Members of the avian family Zosteropidae are renowned for their colonizing ability (Mayr 1945; Mees 1969; Lack 1971) and it has recently been revealed that a South Pacific clade speciated remarkably quickly (Moyle *et al.* 2009). Because of the morphological conservatism noted in the family, Moyle *et al.* (2009) suggested that rapid evolutionary shifts in dispersal ability, rather than adaptive ecological explanations were important for high speciation rates in the family. At a population level therefore, waning gene flow among *Zosterops* populations on different islands may be an important catalyst of divergence. Divergent natural selection on phenotype has been demonstrated in a number of island white-eye populations (Clegg *et al.* 2002*a*, 2008) but the facilitating effects of shifts in dispersal ability and gene flow are not clear.

While the distribution and morphological variation of southwest Pacific avifauna provided the foundation for many early ideas about island speciation (Mayr 1954; Mayr & Diamond 2001), phylogeographic and population genetic studies of the avifauna of this region are only now beginning to accumulate (Kirchman & Franklin 2007; Smith & Filardi 2007; Phillimore *et al.* 2008*b*), lagging behind other well-studied island groups such as the Galápagos (Petren *et al.* 1999), the Caribbean (see Ricklefs & Bermingham (2007) and references therein) and Hawaii (Fleischer & McIntosh 2001). In the Vanuatu archipelago, two *Zosterops* species, the endemic *Z. flavifrons* and the later colonizer *Z. lateralis* have largely coincident distributions, but are at different stages of divergence (Mees 1969; Phillimore 2006; Black 2010). This situation allows us to contrast genetic and phenotypic patterns across the same landscape in related, ecologically similar species that differ in age across islands. We compare regional population structure in the two species, examining the relative importance of gene flow and drift across the archipelago and within-individual island populations. Against the neutral framework describing population connectivity and the geographical factors associated with declining gene flow, we examine patterns of morphological variation to determine whether greater isolation coincides with greater phenotypic divergence.

2. MATERIAL AND METHODS

(a) *Study system*

The Vanuatu archipelago (figure 1) was formed through cycles of volcanic growth and tectonic uplift that began approximately 20–22 Myr ago, although the bulk of current land area was formed in the past 0.5 Myr (Mallick 1975). The islands formed along three volcanic belts, with the oldest islands on the western (Espiritu Santo and Malekula) and eastern belts (Maewo, Pentecost and Efate) and the remaining generally younger islands on the central belt (Mallick 1975). Assuming a relaxed clock model for substitution at mitochondrial protein-coding genes, it appears that the archipelago was colonized somewhere in the region of 2–4 Myr ago by *Z. flavifrons* and less than 0.5 Myr ago by *Z. lateralis* (Phillimore 2006; Black 2010), suggesting that the ancestors of the endemic *Z. flavifrons* colonized before many of the current islands existed. *Zosterops flavifrons* has previously been divided into seven morphological subspecies, which fall into darker and yellow plumage colour groups (Mayr 1945; Mees 1969). Mitochondrial DNA (mtDNA) evidence has cast doubt on the monophyly

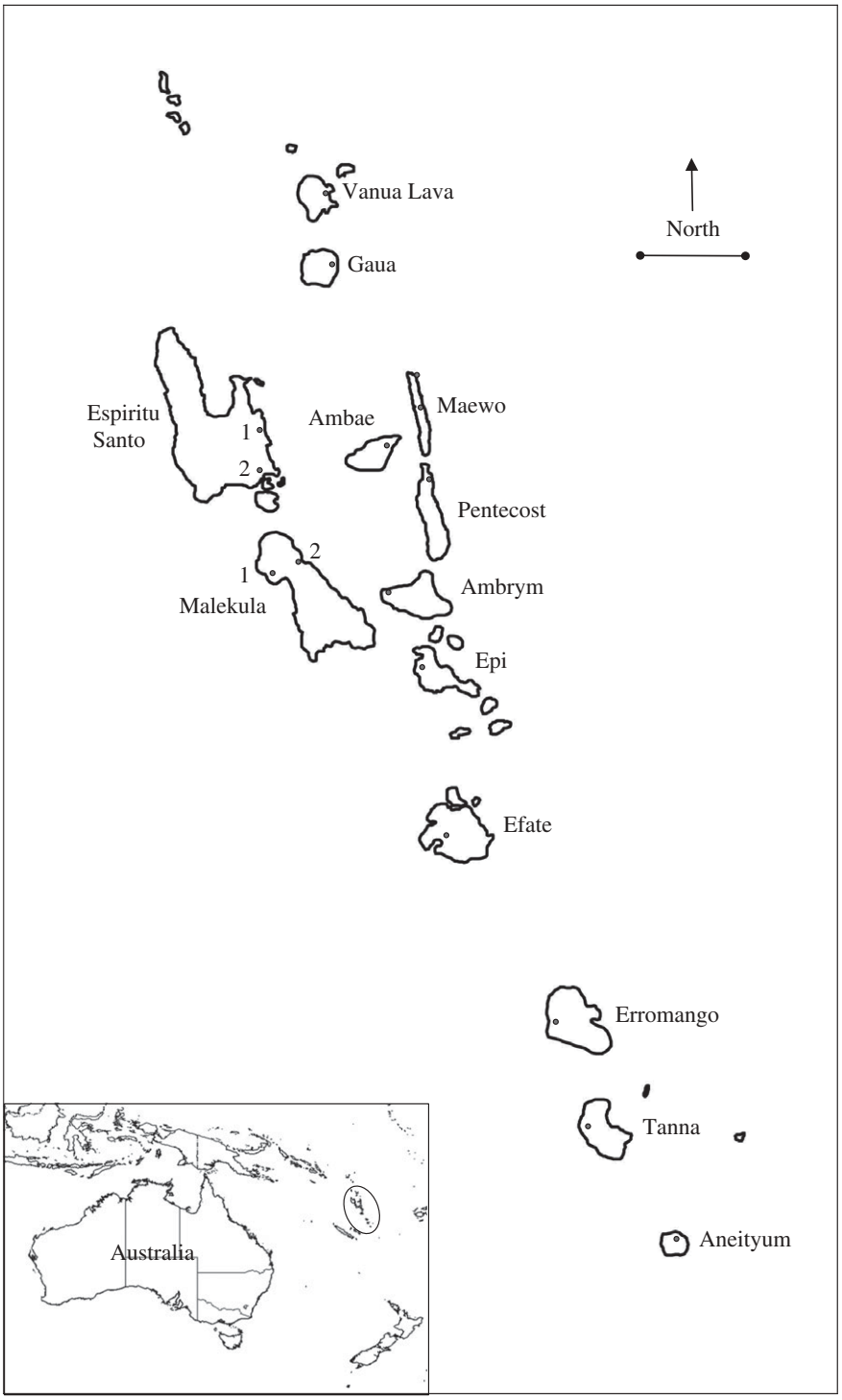

Figure 1. The Vanuatu archipelago showing locations sampled. Numbers refer to locations in table 1. *Zosterops lateralis* were not found on Maewo and Aneityum. Inset shows the position of Vanuatu (circled) in the southwest Pacific. Scale bar, 100 km.

of *Z. flavifrons*, with a single peripheral population possibly representing a cryptic species (Phillimore *et al.* 2008*b*) and the two plumage colour groups possibly resulting from separate invasions (Black 2010). There is also considerable mtDNA structure within each plumage colour group that often does not conform to subspecies designations (Phillimore *et al.* 2008*b*). The deep mtDNA phylogenetic splits within *Z. flavifrons* suggest that gene flow has probably ceased between allospecies of the two plumage groups (Phillimore *et al.* 2008*b*) and we therefore treated them separately in statistical analyses, except where indicated. *Zosterops lateralis* is a prolific island colonizer, with multiple invasions of southwest Pacific islands occurring either directly from its Australian mainland source or via island-hopping (Mayr 1954; Mees 1969; Lack 1971; Clegg *et al.* 2002*b*). In Vanuatu, two to three morphological subspecies of *Z. lateralis* are recognized (Mayr 1945; Mees 1969).

(b) *Sampling*

Birds were caught in mistnets and traps from February to May 2004 (A.B.P.) and February to April 2006 (S.M.C.) (table 1). Morphological measurements

Table 1. Location and sample size information for genetic and morphological analyses. Plumage colour groups for *Z. flavifrons*: D, dark; Y, yellow. Sample size: n_{flav}, *Z. flavifrons*; n_{lat}, *Z. lateralis*.

location (abbreviation)	latitude, longitude	*Z. flavifrons* subspecies	*Z. lateralis* subspecies	sample size		years
				n_{flav}	n_{lat}	
Vanua Lava (Van)	13.88 S, 167.55 E	*perplexa* (D)	*tropica*	51	4	2004, 2006
Gaua (Gau)	14.83 S, 167.67 E	*gauensis* (Y)	*tropica*	24	19 (20[a])	2004
E. Santo, Kole1 (San-1)	15.23 S, 167.16 E	*brevicauda* (D)	*tropica*	32	31	2006
E. Santo, Luganville (San-2)	15.53 S, 167.22 E	*brevicauda* (D)	*tropica*	17	33	2004
Maewo	15.19 S, 168.11 E	*perplexa* (D)	—	18	—	2006
Ambae (Amb)	16.22 S, 167.91 E	*perplexa* (D)	*tropica*	37	3	2006
Pentecost (Pen)	15.47 S, 168.16 E	*perplexa* (D)	*tropica*	9	28	2006
Malekula, Wiawi (Mal-1)	16.13 S, 167.20 E	*macgillivrayi* (D)	*vatensis*	8	3	2006
Malekula, Lakatoro (Mal-2)	16.12 S, 167.42 E	*macgillivrayi* (D)	*vatensis*	13	19	2004
Ambrym (Aby)	15.28 S, 167.99 E	*perplexa* (D)	*vatensis*	61	22	2006
Epi (Epi)	16.45 S, 168.33 E	*perplexa* (D)	*vatensis*	9	9	2004
Efate, Moso Is. (Efa-1)	17.56 S, 168.22 E	*efatensis* (Y)	*vatensis*	34	8	2006
Efate, Port Vila (Efa-2)	17.73 S, 168.32 E	*efatensis* (Y)	*vatensis*	45	25	2004
Erromango (Err)	18.70 S, 169.15 E	*efatensis* (Y)	*vatensis*	17	13	2004
Tanna (Tan)	19.53 S, 169.27 E	*flavifrons* (Y)	*vatensis*	40	12	2004
Aneityum (Ane)	20.33 S, 169.66 E	*majuscula* (D)	—	23	—	2004

[a]Sample size for morphological analysis.

were recorded from each individual (wing, tarsus, culmen length (posterior nostril opening) and culmen depth and width (anterior nostril opening)). Approximately 20–40 µl of blood was collected via venipuncture of the brachial wing vein and stored in 500 µl of 90 per cent ethanol, or absorbed onto a piece of Whatman filter paper (no. 113) wetted with a drop of 0.5 M EDTA (Petren 1998). Samples were individually numbered, and the paper samples dried and stored with a desiccant in a sealed container.

(c) *Molecular methods*

DNA was extracted via an ammonium acetate extraction precipitation method (Nicholls *et al.* 2000). DNA concentrations were estimated on a fluorometer and working dilutions of approximately 20 ng μl^{-1} were prepared. Microsatellite loci isolated from Capricorn silvereye (*Zosterops lateralis chlorocephalus*) (Degnan *et al.* 1999; Frentiu *et al.* 2003) and Seychelles warbler (*Acrocephalus sechellensis*) (Richardson *et al.* 2000) along with the chromo-helicase-DNA-binding (CHD) genes for avian sexing (Griffiths *et al.* 1998) were amplified using standard polymerase chain reaction (PCR) protocols or Qiagen Multiplex PCR kits. Microsatellite amplification of *Z. flavifrons* samples have been described elsewhere (Phillimore *et al.* 2008*b*) and similar protocols were followed for *Z. lateralis* samples. Primer-dependent conditions and loci used for each species are given in the electronic supplementary material, appendix A. Allele size scoring was conducted using the software GeneMapper v. 3.0 (ABI).

(d) *Gene flow/drift models, genetic diversity and geographical associations*

Assumptions of linkage disequilibrium and Hardy–Weinberg equilibrium (HWE) were tested in Genepop 3.2a (Raymond & Rousset 1995). Observed and expected heterozgyosity (H_O and H_E) for each locus/population combination was calculated in Genepop 3.2a (Raymond & Rousset 1995) and averaged across loci for each population. Allelic richness was estimated using the rarefaction method in Fstat v. 2.9.3 (Goudet 1995, 2001) to account for differences in sample size (Leberg 2002). The minimum sample size for rarefaction was eight for *Z. flavifrons* and nine for *Z. lateralis*. All sampled populations were included for *Z. flavifrons*, but two populations with less than five *Z. lateralis* samples (Vanua Lava and Ambae) were excluded.

The correlation between ln-transformed variables of island area and the distance to the nearest island hosting a member of the same group was low for *Z. lateralis* ($r = -0.22$) and the dark plumage group *Z. flavifrons* ($r = 0.12$). We used a multiple regression approach to estimate the degree to which island area and isolation (distance to the nearest island inhabited by a member of the same group) predicted indices of genetic diversity. Each genetic diversity measure (allelic richness, expected heterozygosity and modal *F*; table 2) was considered the dependent variable in turn. Multiple regression was conducted only on *Z. lateralis* and the dark plumage group of *Z. flavifrons* as there was insufficient replication in the yellow plumage group ($n = 4$). For the yellow plumage group we instead ran regressions for each predictor in turn.

To test for an effect of isolation by distance (IBD) within each species across the archipelago, we compared a matrix of pairwise genetic differences ($F_{ST}/(1 - F_{ST})$) (Rousset 1997) with log-transformed geographic distances. A pairwise F_{ST} matrix was calculated in Fstat (Goudet 1995, 2001). Geographic distances were calculated as great circle distances using latitude and longitude co-ordinates in the R software (R Development Core Team 2008). Significance was assessed via a Mantel test, with 10 000 randomizations, and plotted using reduced major

Table 2. Population level genetic variation (A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity) and summary statistics from 2MOD analyses giving the modal values of the posterior distribution of *F* (the probability that two genes in a population are identical by descent and not immigration) and *M* (the number of migrants/generation) for each population. Diversity measures are calculated from 11 loci (*Z. lateralis*) and eight loci (*Z. flavifrons*). Ninety per cent highest posterior density (HPD) ranges are given for *F* and *M* distributions.

location	A_R	H_O	H_E	*F* mode	90% HPD range		*M* mode	90% HPD range	
Z. lateralis									
Vanua Lava	—	0.523	0.503	—	—	—	—	—	—
Gaua	2.82	0.431	0.457	0.1242	0.0751	0.1917	1.59	0.91	2.64
Espiritu Santo	2.25	0.488	0.505	0.1271	0.0845	0.1882	1.50	0.98	2.41
Ambae	—	0.515	0.570	—	—	—	—	—	—
Pentecost	3.07	0.497	0.525	0.0507	0.0267	0.0938	3.44	1.87	7.12
Malekula	3.16	0.525	0.538	0.0059	<0.0001	0.0277	16.27	2.92	119.37
Ambrym	3.32	0.512	0.533	0.0096	0.0008	0.0315	11.71	3.58	59.54
Epi	2.91	0.455	0.496	0.0608	0.0178	0.1375	2.43	0.95	7.76
Efate	2.51	0.342	0.332	0.2810	0.1994	0.3635	0.61	0.40	0.94
Erromango	2.65	0.438	0.428	0.2425	0.1574	0.3465	0.71	0.41	1.19
Tanna	2.27	0.250	0.314	0.4302	0.3255	0.5609	0.29	0.18	0.49
Z. flavifrons (dark plumage group)									
Vanua Lava	3.74	0.446	0.565	0.3896	0.2973	0.3917	0.37	0.24	0.56
Espiritu Santo	3.88	0.513	0.528	0.4617	0.3677	0.5632	0.28	0.18	0.41
Malekula	3.83	0.496	0.557	0.3182	0.2441	0.4042	0.51	0.34	0.73
Epi	3.56	0.568	0.603	0.3635	0.2706	0.4559	0.42	0.27	0.64
Ambae	3.15	0.446	0.473	0.3125	0.2357	0.3917	0.53	0.37	0.77
Maewo	2.80	0.444	0.475	0.4570	0.3792	0.5592	0.28	0.19	0.39
Pentecost	2.71	0.403	0.435	0.3906	0.2967	0.4897	0.37	0.24	0.56
Ambrym	3.13	0.469	0.516	0.3652	0.2933	0.4527	0.41	0.29	0.58
Aneityum	2.26	0.255	0.329	—	—	—	—	—	—
Z. flavifrons (yellow plumage group)									
Gaua	2.26	0.298	0.299	0.3897	0.2851	0.5250	0.33	0.20	0.58
Efate	2.03	0.209	0.231	0.3473	0.2481	0.4526	0.45	0.27	0.70
Erromango	2.85	0.392	0.460	0.1446	0.0676	0.2514	1.14	0.53	2.59
Tanna	2.58	0.385	0.396	0.2083	0.1296	0.3179	0.80	0.45	1.47

axis regression, using web-based software IBDWS (Jensen *et al.* 2005).

We determined the relative contributions of drift versus gene flow across the region for *Z. lateralis* (excluding Ambae and Vanua Lava owing to sample sizes less than 5) and the dark and yellow plumage groups of *Z. flavifrons* considered separately, using the likelihood approach implemented in the program 2MOD (Ciofi *et al.* 1999). Two models were compared: the drift model relates to populations that are subject to drift alone, with no influence from gene flow, and the gene flow/drift equilibrium model (referred to as the gene flow model) relates to a balance between the two microevolutionary forces. The drift model assumes that mutation has not strongly influenced gene frequencies such that alleles are identical by descent, and the gene flow model assumes that the mutation rate is much smaller than the immigration rate (Ciofi *et al.* 1999). Mutational influences on gene frequencies need to be considered, especially in terms of the yellow and dark clades of *Z. flavifrons*, where mtDNA indicates substantial divergence between island populations (0.5–4 Myr, Black 2010). We ran the program four times to ensure convergence of the MCMC algorithm, with 10^5 iterations and the first 10^4 discarded as burn-in. We report the first run of each model after confirming that independent runs yielded similar results. The probability of a model was calculated as the proportion of times that model was supported and also expressed as a Bayes factor (probability model 1/probability model 2). The number of migrants per generation (*M*) for each population was calculated from *F* values according to Ciofi *et al.* (1999). Conditional posterior distributions of *F* (the probability that two genes share a common ancestor) and *M*, were determined from the program Locfit (Loader 2007) within the R framework (R Development Core Team 2008) using values from only the favoured model. The mode and 90 per cent highest posterior density (HPD) limits were calculated in R using code modified from Lopez-Vaamonde *et al.* (2006).

(e) *Population genetic structure and connectivity patterns within each species*

To more fully explore the population genetic structure of *Z. lateralis*, we used Bayesian clustering methods implemented in Structure v. 2, to assess genetic structure without using prior geographical information (Pritchard *et al.* 2000). The number of genetic clusters (*k*) suggested by the data without using prior population information was evaluated for values of *k* between 1 and 9, using five independent runs at each value of *k*, each with a burn-in length of 10^5 and run length of 10^6 iterations. The default program settings were used, including correlated allele frequencies (Falush *et al.* 2003) and an admixture model

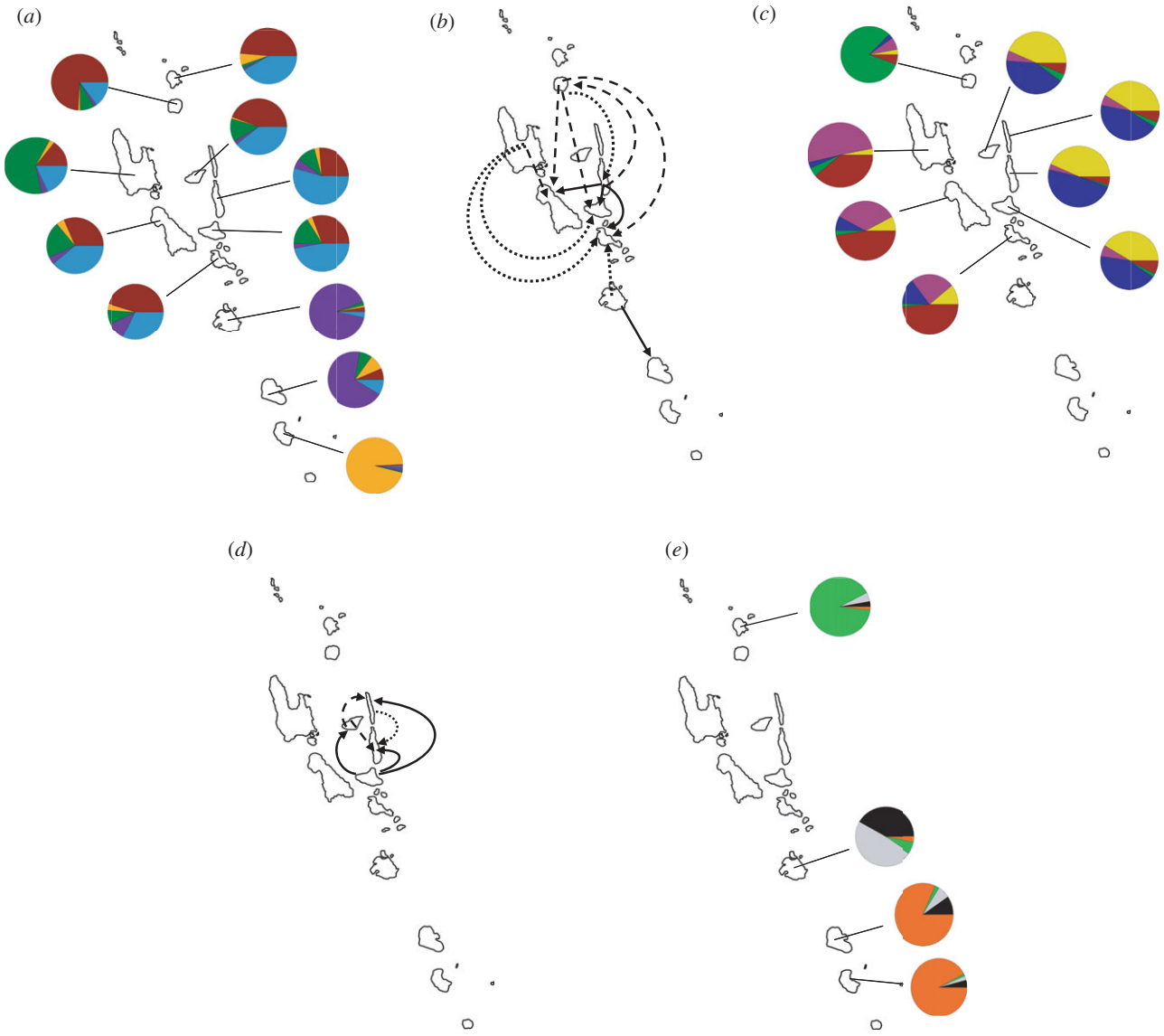

Figure 2. Distribution of microsatellite genetic clusters from STRUCTURE and estimates of migration rates from BAYESASS among islands for (*a*) *Z. lateralis*: five genetic clusters. In text, cluster 1, red; 2, yellow; 3, green; 4, purple; 5, blue. (*b*) *Zosterops lateralis* migration rates; (*c*) dark plumage group *Z. flavifrons* clusters; (*d*) dark plumage group *Z. flavifrons* migration rates; and (*e*) yellow plumage group *Z. flavifrons* genetic clusters. Note that STRUCTURE analysis was conducted for each group separately and pie colours do not relate to across-group comparisons. STRUCTURE data for *Z. flavifrons* taken from Phillimore *et al.* (2008*b*). Solid lines show migration rates more than 0.1, dashed lines 0.03–0.1 and dotted lines 0.02–0.03.

allowing individuals to have mixed ancestry. The prior probabilities of the best run for each value of k (i.e. the run with the smallest value of $-2\log \Pr(X|k)$) were compared to identify the most likely number of genetic clusters. Individual assignments to clusters were identified from the output of the best run for the most likely value of k. STRUCTURE identifies groups of individuals at the uppermost hierarchical level and when there is uneven migration among populations, such as that evidenced by IBD relationships, more subtle nested sub-structuring may be overlooked (Evanno *et al.* 2005). Therefore, following assignment of individuals to each of the clusters identified in the first STRUCTURE analysis, separate analysis was carried out on those clusters that were geographically widespread, to determine if there was sub-structuring within clusters.

A STRUCTURE analysis of each *Z. flavifrons* plumage group has previously been reported (see Phillimore *et al.* (2008*b*) for details of analysis). For comparison with *Z. lateralis*, a summary of cluster associations within each *Z. flavifrons* plumage group is presented in figure 2.

Contemporary inter-island migration rates for *Z. lateralis* populations and a subset of *Z. flavifrons* populations were estimated via the Bayesian method implemented in BAYESASS 1.3 (Wilson & Rannala 2003). This method simultaneously estimates recent migration rates (i.e. the fraction of individuals within a population that are migrants per generation) along with a suite of other parameters including individual migrant ancestries (Wilson & Rannala 2003). The performance of the method as assessed by Faubet *et al.* (2007) was found to be accurate under conditions of moderate genetic differentiation ($F_{ST} \geq 0.05$) and small migration rates (no more than one-third of the individuals in a population being migrant per

generation). For *Z. lateralis*, the two populations with low sample size (Ambae and Vanua Lava) were excluded from the analysis. The BayesAss analysis was inappropriate for most of the *Z. flavifrons* populations, given that many of the islands were found to be monophyletic and in some cases highly divergent on a mtDNA gene tree (Epi, Santo, Tanna, Vanua Lava; Phillimore *et al.* 2008*b*), which is consistent with an absence of gene flow. In the case of the Gaua and Efate populations of the yellow *Z. flavifrons*, ongoing gene flow seems improbable given the large distance between these two islands. Moreover, pairwise F_{ST} estimates for many of these island populations were large (more than 0.3; Phillimore *et al.* 2008*b*). The one group of populations where ongoing gene flow does appear plausible on the basis of low F_{ST} values (Phillimore *et al.* 2008*b*) is for dark group *Z. flavifrons* members inhabiting Ambae, Ambrym, Maewo and Pentecost in the eastern part of central Vanuatu. Consequently, we considered these four islands in a BayesAss analysis. We set delta values for allele frequencies, inbreeding coefficients and migration rates such that acceptance rates for changes in these parameters fell between 40 and 60 per cent (Wilson & Rannala 2003). The program was run for 21×10^6 iterations including a burn-in of 2×10^6 iterations. Model convergence was assessed by comparison of posterior probability densities of inbreeding coefficients and allele frequencies across 10 replicate runs (five replicates for the *Z. flavifrons* analysis) with different starting seeds (Wilson & Rannala 2003). Distributions of log-likelihood values for each converged run were compared to determine the best run from which to obtain parameter estimates.

(f) *Morphological variation*

Analysis of morphological data was conducted in the R framework (R Development Core Team 2008). Between-measurer repeatability (between S.M.C. and A.B.P.) was assessed from measures of museum specimens of *Z. flavifrons* and *Z. lateralis* (from the Museum of Natural History, Tring) for culmen traits, and wild-caught blue tits (*Cyanistes caeruleus*) for wing, tail and tarsus, following the procedure described in Phillimore *et al.* (2008*b*). Measurements were compared directly, with the exception of culmen length, where a systematic difference in measurement was apparent. Correction factors of 0.048 and 0.064 were added to the ln-transformed culmen length measurements made by A.B.P. for *Z. lateralis* and *Z. flavifrons*, respectively, prior to calculating repeatability. Traits with high between-measurer repeatability were included in the analysis (wing: repeatability (r) = 0.84, sample size (n) = 25; tarsus: $r = 0.9$, n=25; culmen length $r = 0.86$, $n = 46$; culmen depth $r = 0.87$, $n = 46$, culmen width $r = 0.69$, $n = 46$). Tail length was removed from further analysis owing to lower between-measurer repeatability ($r = 0.58$, $n = 25$).

Principal components analysis was conducted on log-transformed wing, tarsus, culmen length, culmen depth and culmen width measurements. We also calculated relative wing length as the residual values from a regression of log-transformed wing length on log-transformed tarsus length. Latitudinal effects on morphology, summarized as (i) principal components and (ii) relative wing length, were tested using least squares regression on mean location values (note that we did not make any correction for phylogenetic similarity or levels of population connectivity).

Correlation between total (measured) phenotypic variance and covariance, measured as the sum of variances for each morphological trait in each population, and island isolation and area were assessed using multiple regression. For *Z. flavifrons*, population age estimates obtained from coalescence estimates of mtDNA lineages, assuming a mean substitution rate of 2 per cent per million years, were available (Phillimore *et al.* 2008*b*). Therefore, median age (measured as the median age of the most recent common ancestor shared between a focal island population and its closest relative on the maximum clade credibility tree) was included as a covariate in addition to island isolation and area for *Z. flavifrons*. Lack of coalescence of mtDNA lineages precluded a similar analysis in *Z. lateralis* (Phillimore *et al.* 2008*b*).

Pairwise multivariate morphological differences between populations for both species were quantified via a MANOVA-based approach (described in Phillimore *et al.* 2008*b*) as the proportion of the total measured phenotypic variation between and within the two populations, that was found at the between population level. Note that this is identical to calculating multivariate P_{ST} (Leinonen *et al.* 2006)—itself a phenotypic equivalent of Q_{ST} (Spitze 1993)—under the assumption that all phenotypic differences between populations are due to additive genetic variance and a within-population heritability of 0.5.

Correlations between phenotypic and genetic (F_{ST}) matrices, and phenotypic and geographical distance matrices, were assessed using Mantel tests with 10 000 permutations. The pairwise F_{ST} matrix obtained from Fstat (Goudet 1995, 2001) was first standardized to account for differences in within-population variability (Hedrick 2005) using methods and programs described in Meirmans (2006).

3. RESULTS

(a) *HWE and linkage disequilibrium*

Eleven microsatellite loci were screened for *Z. lateralis*. Two populations had a deficit of heterozygotes: Ambrym (p-value combined across loci, $p = 0.01$) and Tanna ($p = 0.007$). In both cases, this was because of a small subset of loci and therefore heterozygote deficiency was not a population-specific problem. Two loci had a deficit of heterozygotes: ZL45 (p-value combined across populations, $p = 0.05$) and ZL38 ($p < 0.001$). However, this does not suggest a locus-specific problem with null alleles, as in both of these cases only one and three of the 12 populations, respectively, were responsible for the significance of the combined value. Linkage disequilibrium was not detected for any locus pair ($p > 0.06$ for all pairwise comparisons). Therefore, all 11 loci were retained for further analysis of variation in *Z. lateralis* populations. Eight loci were used to

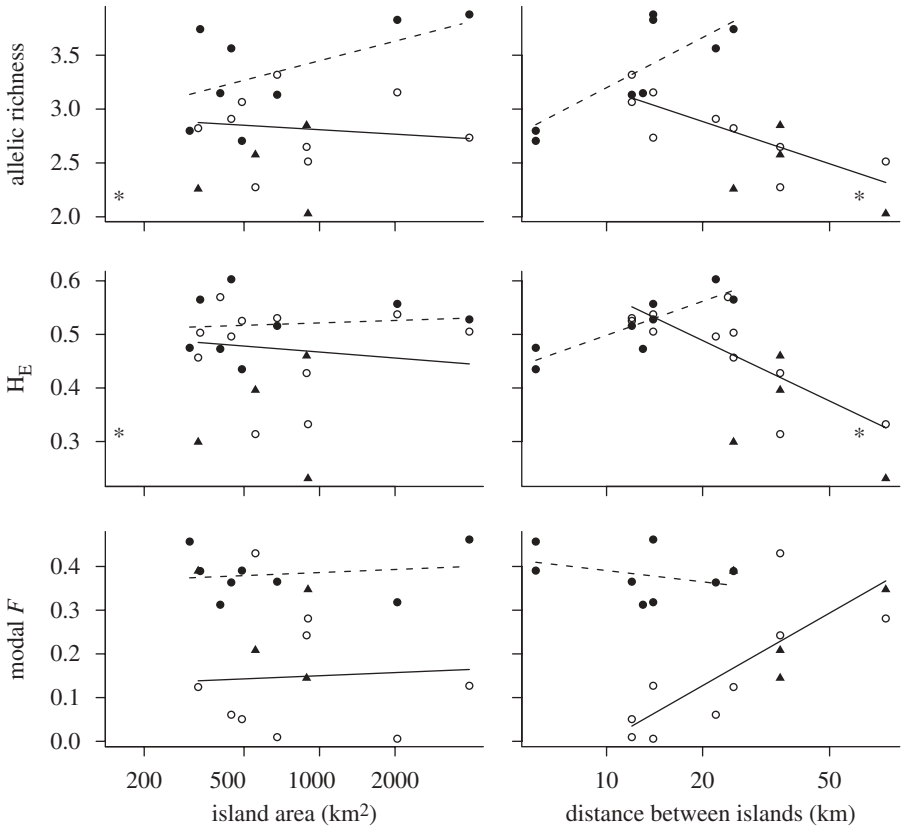

Figure 3. Regression of three diversity measures (allelic richness, expected heterozygosity (H_E) and modal *F* values from 2MOD analysis) with island area (square kilometres) and distance to nearest inhabited island (kilometres) (of the same colour morph in the case of *Z. flavifrons*). *Zosterops lateralis*: open circles, solid line; dark plumage *Z. flavifrons*: black circles, dashed line; yellow plumage *Z. flavifrons*: black triangles (no regression shown). *Zosterops flavifrons* from Aneityum (indicated by an asterisk) was not included in regressions because of its paraphyletic status.

quantify microsatellite variation in *Z. flavifrons*. The use of these eight loci conformed to assumptions of HWE and linkage equilibrium, and across loci and populations (see Phillimore *et al.* (2008*b*) for details of assumption testing).

(b) *Gene flow/drift models, genetic diversity and geographical associations*

In *Z. lateralis*, an increase in island isolation was significantly associated with a decrease in allelic richness and heterozygosity and an increase in the inbreeding coefficient (figure 3 and table 3). In the darker *Z. flavifrons* group, allelic richness increased significantly with island area, but correlations of area with other indices of genetic diversity were not significant. A counterintuitive trend in the dark *Z. flavifrons* was that distance to the nearest island correlated positively with both allelic richness and expected heterozygosity. None of the single predictor models involving the yellow *Z. flavifrons* returned a significant correlation.

A significant IBD relationship was found for *Z. lateralis* (Mantel test: $Z = 25.01$, $r = 0.57$, $p = 0.002$; figure 4*a*). This relationship was strongly influenced by the most isolated and differentiated southern population on Efate, Erromango and Tanna (excluding these populations, $Z = 3.44$, $r = 0.12$, $p = 0.344$; figure 4*a*). In *Z. flavifrons*, an IBD pattern had borderline significance across the entire archipelago ($Z = 64.25$, $r = 0.33$, $p = 0.05$; figure 4*b*), while significant relationships were found for each plumage group considered separately (dark plumage group: $Z = 7.66$, $r = 0.44$, $p = 0.02$; yellow plumage group: $Z = 6.59$, $r = 0.93$, $p < 0.0001$; figure 4*c*).

Results from 2MOD analysis supported a gene flow-drift equilibrium model over a drift-alone model in *Z. lateralis* (p(gene flow) = 0.9998, Bayes factor = 4999). Lower modal *F* values were found for central populations of Pentecost, Malekula, Ambrym and Epi, translating into particularly high number of migrants/generation for Malekula and Ambrym. In contrast, higher *F* values were characteristic of more peripheral islands (Gaua, Efate, Erromango and particularly Tanna) along with the largest island (Espiritu Santo; table 2). A gene-flow/drift equilibrium model was also favoured in both plumage groups of *Z. flavifrons* (p(gene flow) = 1 for each); however, individual populations tended to have high modal *F* values, indicative of drift, with correspondingly low estimates of number of immigrants per generation (table 2).

(c) *Population genetic structure and connectivity patterns within each species*

The STRUCTURE analyses indicated that *Z. lateralis* was composed of five genetic clusters (k; probability of 5 clusters = 1). All other tested values of k were not supported and had probabilities approaching zero. The average assignment probabilities of individuals to each cluster were reasonably high (average assignment probability for individuals in cluster 1 = 0.71, cluster

Table 3. Intercepts and slopes from multiple regression showing relationships between diversity and inbreeding indices with island area and distance to the nearest island.

	Z. lateralis	dark *Z. flavifrons*	yellow *Z. flavifrons*[a]
allelic richness (A_R)			
intercept	4.56 ± 0.90***	0.06 ± 0.44	i = 3.82 ± 1.75; ii = 1.55 ± 3.36
distance	−0.43 ± 0.13* [0.63]	0.67 ± 0.10** [0.56]	−0.38 ± 0.22 [0.24]
area	−0.06 ± 0.11 [0.02]	0.26 ± 0.06** [0.27]	0.137 ± 0.523 [0.03]
heterozygosity (H_E)			
intercept	0.96 ± 0.20**	0.25 ± 0.10	i =0.75 ± 0.49; ii = 0.13 ± 0.96
distance	−0.12 ± 0.03** [0.65]	0.09 ± 0.02* [0.72]	−0.11 ± 0.13 [0.26]
area	−0.02 ± 0.02 [0.02]	0.01 ± 0.01 [0.01]	0.04 ± 0.15 [0.02]
modal *F*			
intercept	−0.59 ± 0.41	0.41 ± 0.19	i =0.17 ± 0.64; ii = 1.08 ± 0.94
distance	0.18 ± 0.06* [0.59]	−0.04 ± 0.04 [0.12]	0.03 ± 0.17 [0.01]
area	0.01 ± 0.05 [0]	0.01 ± 0.03 [0.03]	−0.13 ± 0.15 [0.27]

Significance from zero indicated by:
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a]Intercepts and slopes reported for yellow plumage group *Z. flavifrons* are from univariate regression. The two intercepts (i and ii) are for distance and area, respectively. Partial r^2 and r^2 (in the case of the yellow *Z. flavifrons*) are reported in brackets.

2 = 0.91, cluster 3 = 0.77, cluster 4 = 0.88, cluster 5 = 0.64). The genetic clusters showed geographic affinities, with members of cluster 3 predominantly found on Espiritu Santo and represented in lower frequencies on neighbouring islands, members of clusters 1 and 5 being more widely distributed across islands at the northern end of the archipelago, members of cluster 4 found predominantly on Efate and Erromango, and members of cluster 2 on the southern island of Tanna (figure 2*a*). Separate STRUCTURE analysis of each of the three most widely distributed genetic clusters (1, 3 and 5) did not reveal further structure at lower levels, with each cluster comprising a single genetic group when treated separately (values tested: $k = 1–5$ for each cluster, with the same settings as the initial STRUCTURE analysis).

The assignments of a similar STRUCTURE analysis applied separately to the dark and yellow populations are shown in figure 2*c*,*e* (protocol described in Phillimore *et al*. 2008*b*). Five clusters were identified for the dark plumage group and four for the yellow group. Based on the proportions of the populations belonging to each cluster, it is clear that in the dark plumage group Epi, Malekula and Santo have similar compositions as do Ambae, Ambrym, Maewo and Pentecost, with Vanua Lava being quite distinct. In the case of the yellow plumage group, Erromango and Tanna have similar compositions, while Efate and particularly Gaua are quite distinct.

Of the 10 independent BAYESASS runs conducted to quantify degree and direction of migration rates among *Z. lateralis* populations, eight converged on a similar solution. The best of the eight runs was identified from the distribution of log-likelihood values. Figure 2*b* displays all migration rate estimates above 0.02. The predominant direction of migration was north to south, with generally low levels of upstream migration (from south to north). Gaua, Espiritu Santo, Pentecost, Efate and Tanna each had a high proportion of non-migrants (electronic supplementary material, appendix B). The central islands of Malekula, Ambrym and Epi had high (more than 0.15) immigration rates from Pentecost in particular, as well as influences from Espiritu Santo and Gaua. Epi was additionally affected by northward gene flow from Efate. Erromango had a high migration rate from neighbouring Efate (figure 2*b*; electronic supplementary material, appendix B). Migration rates out of Espiritu Santo into Gaua, Malekula, Ambrym, Epi and Erromango all exceeded 0.01; however, migration rates into Espiritu Santo from other islands never exceed this value (electronic supplementary material, appendix B).

All five independent BAYESASS runs for the eastern four populations of the dark *Z. flavifrons* group converged on a similar solution. Ambrym had the highest proportion of non-migrants and contributed a substantial proportion of migrants to the three other populations. Pentecost was a sink population, and both Ambae and Maewo were the source of a small amount of migration to neighbouring islands (figure 2*d*; electronic supplementary material, appendix C).

(d) *Morphological variation*

In *Z. lateralis*, three principal components (PCs) summarized 77.2 per cent of variation in five morphological traits. High loading coefficients of similar size and the same sign for each trait at PC1 (explaining 44.6% of variance) indicated that this component represents overall size variation and is henceforth referred to as body size. Structure coefficients at PC2 (18.4% of variance) contrasted culmen length with culmen width, and are referred to as culmen shape; PC3 (14.1% of variance) contrasted tarsus length and culmen length, and is referred to as body shape. In *Z. flavifrons*, three PCs summarized 82.4 per cent of variation. PC1 corresponded to body size (54.8% of variance), PC2 (16.2% of variance) contrasted culmen depth and width with wing, tarsus and culmen length measures and is

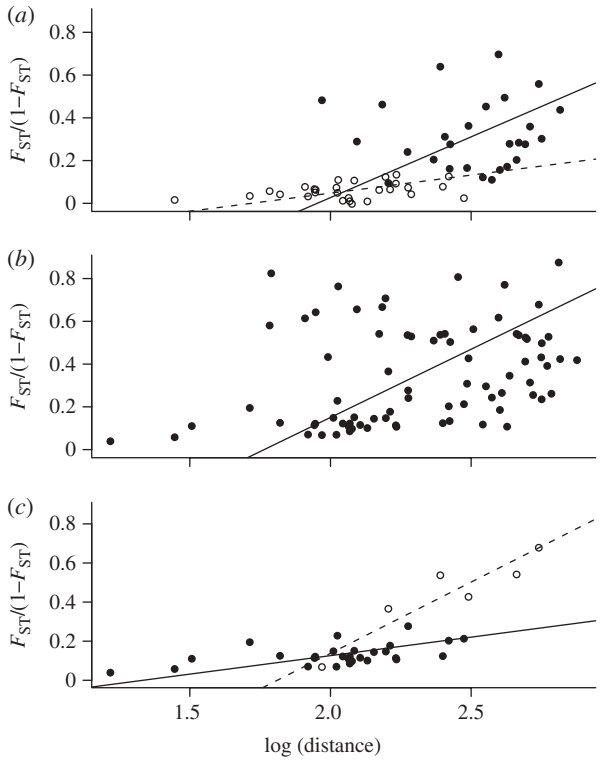

Figure 4. Isolation by distance relationships for *Zosterops*. (*a*) *Zosterops lateralis*: pairwise comparisons among all islands (solid line shows the reduced major axis (RMA) regression line when all points (open and closed circles) are included), and pairwise comparison among northern islands only (from Epi northwards to Vanua Lava, open circles only, dashed RMA line regression line). (*b*) *Zosterops flavifrons*: pairwise comparisons among all island populations. (*c*) *Z. flavifrons*: pairwise comparisons within yellow (open circles, dashed RMA regression line) and dark (closed circles, solid RMA regression line) plumage groups.

referred to as body shape, and PC3 (11.4% of variance) contrasted culmen width with culmen depth and is referred to as bill shape.

Some latitudinal trends in morphology were evident in both species. In *Z. lateralis*, relative wing length increased with latitude for *Z. lateralis*: ($r^2 = 0.47$, $p < 0.01$) and there was also a marginally non-significant trend for an increase in overall body size ($r^2 = 0.22$, $p = 0.09$). Bill shape and body shape in *Z. lateralis* did not display latitudinal patterns (bill shape: $r^2 = 0.07$, $p > 0.3$; body shape: $r^2 = 0.17$, $p = 0.1$). In contrast, *Z. flavifrons* populations did not show latitudinal patterns in either relative wing length ($r^2 = 0.12$, $p > 0.1$) or body size ($r^2 = 0.08$, $p > 0.2$). Body shape (PC2) in *Z. flavifrons* was associated with latitude, more southerly populations having longer wings, tarsi and bills but narrower and shallower bills (PC2 and latitude: $r^2 = 0.36$, $p < 0.05$).

Total phenotypic variance in a population was not related to island area or level of isolation for either species, or to population age for *Z. flavifrons* (table 4). Average pairwise phenotypic divergence among populations were: *Z. lateralis* 0.69 (range 0.21–0.96); *Z. flavifrons* dark plumage group 0.70 (0–0.98), *Z. flavifrons* yellow plumage group 0.81 (0.50–0.95), all *Z. flavifrons* (excluding Aneityum) 0.71 (0–0.98). Phenotypic divergence among

Table 4. Multiple regressions of total phenotypic variance with island area, distance to nearest island (isolation) and population age. For *Z. flavifrons* plumage groups, distance was measured to nearest island of the same plumage group (excluding the population from Aneityum). Limited degrees of freedom for the yellow plumage *Z. flavifrons* group precluded the inclusion of three covariates simultaneously; therefore the relationship between total phenotypic variance and age was calculated separately.

component	estimate (±s.e.)	*t*-value	*p*-value
total phenotypic variance			
Z. lateralis			
island area	$<0.001 \pm 0.002$	0.09	>0.9
distance	-0.003 ± 0.002	−1.34	>0.2
Z. flavifrons (all populations)			
island area	0.001 ± 0.001	1.03	>0.3
distance	-0.001 ± 0.001	−0.97	>0.3
age	0.001 ± 0.002	0.40	>0.7
Z. flavifrons (dark plumage group)			
island area	$<0.001 \pm 0.001$	0.15	>0.8
distance	-0.002 ± 0.001	−1.64	>0.1
age	0.004 ± 0.003	1.37	>0.2
Z. flavifrons (yellow plumage group)			
island area	0.002 ± 0.002	0.95	>0.5
distance	$<-0.001 \pm 0.001$	−1.74	>0.8
age	0.002 ± 0.006	0.30	>0.7

populations did not exhibit an IBD pattern in *Z. lateralis* (Mantel test of phenotypic divergence versus log-transformed geographical distance: $r = 0.239$, $p = 0.15$); however, a marginally non-significant relationship was found for *Z. flavifrons* ($r = 0.24$, $p = 0.07$). This relationship was not apparent when each plumage group was considered separately (dark plumage group: $r = 0.13$, $p > 0.2$; yellow plumage group: $r = 0.22$, $p > 0.2$).

Pairwise phenotypic divergence and standardized F_{ST} values were not correlated in *Z. lateralis* (Mantel $r = 0.4$, $p > 0.1$). A marginally non-significant correlation between phenotypic and genetic divergence matrices was found across all *Z. flavifrons* populations ($r = 0.29$, $p = 0.06$), but when the dark and yellow plumage groups were considered separately, no such relationship was evident (dark plumage group: $r = -0.07$, $p > 0.6$; yellow plumage group: $r = 0.07$, $p > 0.3$).

4. DISCUSSION

Bird species that colonize archipelagos are generally believed to be stronger dispersers (Diamond *et al.* 1976). However, once on an archipelago, selection may lead to reduced dispersal ability (e.g. McCall *et al.* 1998), and as a consequence, migration between populations on geographically separated islands is expected to decline over time (Mayr & Diamond 2001). Changes in the rates of gene flow in turn have the potential to influence rates of phenotypic divergence (Price 2008). In our examination of genetic and morphological diversity in two congeneric, co-distributed bird species at different stages of population divergence across the Vanuatu archipelago, we

observed different regional population genetic structures that possibly reflect the transition from a gene flow-mediated system towards a drift-mediated system in an archipelago situation. The development of complex and asymmetrical patterns of gene flow in the more recent colonizer gave way to a situation of genetically isolated, drift-influenced populations within groups of the endemic species. Patterns of phenotypic variance and divergence were not strongly related to levels of gene flow or geographic isolation in either species. Phenotypic traits were weakly associated with latitude, consistent with climate-related influences on phenotype, whether through selection or phenotypic plasticity.

(a) *Constraints on gene flow and drift: the influence of island isolation and area*

There were marked differences in regional population genetic structure between the *Zosterops* species. Among populations of the more recent colonizer, *Z. lateralis*, gene flow maintained a strong influence on population structure at both the regional level and within many of the individual populations. However, frequent long-distance movements (resulting in gene flow), expected if dispersal was maintained at the 'colonizing' level, were not apparent. Gene flow was constrained by increasing levels of island isolation, producing distinctive patterns of reduced genetic diversity and increasing genetic isolation. The importance of island isolation on genetic characteristics was further shown by the relatively elevated inbreeding coefficients (*F* values) observed in peripheral populations (Efate, Erromango and Tanna in the south, Vanua Lava and Gaua in the north) compared with central *Z. lateralis* populations. The higher likelihood that alleles were identical owing to descent in the peripheral island populations demonstrated that they represent more 'closed' populations. However, the central and largest island of Espiritu Santo also had a large *F*-value which we infer is the product of drift, and thus geographical isolation provides only a partial explanation of the level of genetic isolation. Results from the Bayesian analysis of migration show that Espiritu Santo was a source area, sending out migrants to surrounding islands, but receiving them at a much lower rate, which is the expected pattern of migration between densely and more sparsely populated areas (Lenormand 2002). Therefore, despite its larger size and central location, asymmetrical migration may leave the Espiritu Santo population exposed to drift-mediated divergence.

Despite having occupied the archipelago for a much longer evolutionary time period (Phillimore *et al.* 2008*b*; Black 2010), *Z. flavifrons* populations showed only a partial shift to a drift-modulated system, with a general lack of significant relationships between island area and indices of diversity/inbreeding in both the dark and yellow groups. Only one relationship, that of increasing allelic richness and area (used as a proxy for population size) for dark forms of *Z. flavifrons*, was significant and in the direction predicted for a drift-mediated system, suggesting that increased population size buffers the largest populations from allele loss via drift to some extent. The corresponding lack of any significant distance–diversity patterns (neither allelic richness nor heterozygosity were significantly negatively affected by increasing island isolation, and in fact the trend in the dark plumage group was one of increasing diversity with isolation) is consistent with a shift away from a gene flow-mediated system.

Although a gene flow/drift model was highly favoured over a drift-alone model in *Z. flavifrons* plumage groups, the influence of drift on individual island populations was pronounced (much more so than for any *Z. lateralis* population), and was equally evident in both central and peripheral islands. A similar pattern in red deer populations was interpreted as evidence of a shift from a gene flow/drift equilibrium system towards a drift system that had not been completed owing to the historical time frame of population fragmentation (Kuehn *et al.* 2003). The time frame available for population fragmentation in *Z. flavifrons* may be complicated by (i) the possibility that the current geography of the archipelago was not fully developed when the ancestors of *Z. flavifrons* colonized (Mallick 1975); (ii) that multiple independent colonizations seem probable (Black 2010); and (iii) that background extinction of island populations may be high (Ricklefs & Bermingham 1999). Given that age estimates for *Z. flavifrons* populations are more than 0.5 Myr in 10 of the 13 populations and most of the populations are unlikely to be connected via current gene flow (Black 2010), we suggest that the incomplete transition to a signature of a drift-mediated system for *Z. flavifrons* plumage groups possibly reflects low levels of ancient gene flow among populations of the same plumage group, with some contemporary gene flow among the eastern islands, rather than a lack of time.

Results from the two *Zosterops* species contrast with studies of island taxa that were marooned on islands formed by rising sea levels. For example, drift was identified as the main determinant of regional population structure in lava lizards, *Microlophus albemarlensis* complex, on the islets in the Galápagos (Jordan & Snell 2008) and Australian bush rats, *Rattus fuscipes greyii*, on South Australian islands (Hinten *et al.* 2003). Even in species that must have been capable of over-water colonization, a strong signature of drift has been reported to develop rapidly (e.g. Galápagos hawk, *Buteo galapagosensis*; Bollmer *et al.* 2005). The maintenance of dispersal capacity in *Z. lateralis* and to a lesser extent within some populations of *Z. flavifrons* may not be unusual for birds inhabiting the Vanuatu archipelago. A phylogeographic study of mtDNA variation in three other widespread birds (buff-banded rail, *Gallirallus phillippensis*; emerald dove, *Chalcophaps indica*; and streaked fantail, *Rhipidura spilodera*) across Vanuatu revealed little evidence for geographical structure, suggesting a role for ongoing gene flow (Kirchman & Franklin 2007).

(b) *Population connectivity in* Z. lateralis *and* Z. flavifrons *populations*

Population connectivity patterns in *Z. lateralis* offer insights into geographical influences during the early stages of diversification in the archipelago, and

highlight the complexities of movement patterns across a fragmented environment. The highest levels of gene flow occurred among neighbouring central islands, with some indication that stepping-stone rather than direct dispersal may operate in some cases (e.g. Espiritu Santo to Ambrym via Malekula). Patterns of gene flow inferred from the BayesAss analysis (proportion of migrants per generation) compared with estimates calculated from *F* values in 2MOD (number of migrants per generation) were broadly congruent with respect to identifying those islands subject to high levels of gene flow (some central islands) compared with peripheral islands that are isolated, e.g. Tanna. However, the BayesAss analysis has the advantage of showing direction and asymmetries in gene-flow patterns.

Asymmetrical gene flow among islands is a feature of the system, even among the neighbouring central islands. There are a number of possible explanations for the development of the particular source–sink relationships seen in *Z. lateralis*. First, uneven patterns of gene flow could be because of differences in population productivity (Lenormand 2002). If we take island size as a proxy for population size and productivity, the evidence for this explanation in *Z. lateralis* is equivocal, with representatives of both larger and smaller islands appearing to be source and sink populations. Second, dispersal ability or inclination may vary among populations. It is not obvious what combination of population, ecological and/or morphological differences could produce dispersal variation patterns that would account for the observed source–sink relationships among populations of *Z. lateralis*. However, the extreme variation noted in the dispersal ability of different *Z. rendovae* subspecies within the Solomon archipelago (Mayr & Diamond 2001) indicates that dispersal variation may be an important feature of fragmented *Zosterops* populations.

Taking an archipelago-wide view, the direction of gene flow was predominantly north to south. This is consistent with the dominant pattern noted from species distributions in the broader southwest Pacific region (i.e. New Guinea, Bismarks, Solomons, Vanuatu, Fiji)—that of 'downstream' avian colonization routes from regions of higher to lower biodiversity (Diamond *et al.* 1976; Mayr & Diamond 2001). Interestingly, this general pattern is at odds with the latitudinal trends in relative wing length, as in both species, southern populations possessed longer relative wing lengths, and presumably have greater dispersal ability (Skjelseth *et al.* 2007). One possible resolution of the discord between predominant dispersal direction and latitudinal trends in wing length is the idea that colonization itself creates a selection filter (e.g. Berry 1998); thus only the strongest flyers would reach the most southerly islands. No single explanation accounts for the observed source–sink patterns; however, a combination of the dominant pattern (north–south dispersal), overlaid with population density influences and other population-level idiosyncrasies such as dispersal inclination, may all contribute.

In addition to the ongoing distance-limited gene flow, the population genetic structure of *Z. lateralis* may be impacted by local extinctions and recolonizations that would have the effect of weakening the development of geographical structure (Slatkin 1987). Diamond & Marshall (1977) discussed the changing distributions of Vanuatu avifauna since the beginning of ornithological expeditions in 1774, with both expansions and retractions of previously recorded distributions, particularly among the central islands from Epi to Espiritu Santo and Maewo. *Zosterops lateralis* was previously recorded on Maewo (Bregulla 1992), but appears to be currently absent or extremely rare (S. Totterman, VanBirds 2005, personal communication; S.M.C., personal observation, 2006). In all likelihood, Maewo will be recolonized from a neighbouring island given its geographical proximity (less then 6 km) to large populations of *Z. lateralis*. However, despite this example, extinction and recolonization events within *Z. lateralis* do not appear to have been frequent enough to impede the development of structured variation as evidenced by IBD patterns, the discernible geographical structure of neutral alleles—even among groups of central islands—and the substantial divergence of particular populations, e.g. Tanna. Phylogenetic information will need to be used to determine if this remains true over longer evolutionary time frames, thus fulfilling the 'population persistence' requirement for divergence and potential adaptive radiation (Diamond & Marshall 1977; Ricklefs & Bermingham 2007).

Among *Z. flavifrons*, highly structured genetic clusters reflect the long history of isolation that many of the populations, even within plumage groups, appear to have experienced. Among the *perplexa* subspecies inhabiting Ambae, Maewo, Pentecost and Ambrym, some gene flow occurs. Like the patterns in *Z. lateralis*, gene flow is asymmetrical, with Ambrym in the south being the primary source population. Again, explanations for asymmetrical gene flow across this small region cannot be pinpointed to one factor, but presumably are due to a combination of population density differences and local exctinction–recolonization dynamics. The estimates of gene flow from 2MOD serve to show that connectivity across *Z. flavifrons* populations is very low, although actual estimates should be treated with caution owing to the influence that mutation may have on gene frequencies (Ciofi *et al.* 1999).

(c) *Gene flow, phenotypic divergence and factors limiting avian divergence in Vanuatu*

The rapid rate of lineage divergence found for *Zosterops* combined with overall morphological conservatism within the family led Moyle *et al.* (2009) to argue that allopatry plays an important role in the generation of lineage diversity in this group. At a population level, we did not find evidence to suggest that waning gene flow was associated with increased phenotypic divergence among the *Z. lateralis* populations. Further, phenotypic variation did not mirror the negative relationship between genetic diversity and increasing island isolation that would be expected if gene flow also impacted levels of phenotypic diversity. Petren *et al.* (2005) likewise found little evidence of a constraining role of gene flow in Darwin's finches. If

phenotypic differences between the *Zosterops* populations on different islands have a genetic basis (i.e. they are not the product of phenotypic plasticity alone), then the lack of relationship between neutral genes and phenotype implies that selection is important in allopatric divergence. Indeed, morphological patterns showed latitudinal trends, with body size and relative wing length increasing at higher latitude, which is consistent with local climate generating natural selection either directly or indirectly via its effect on habitat. For this study, it was not logistically feasible to test if variation between populations has a genetic basis, as translocation or common garden experiments are required. However, a growing list of translocation experiments in birds report that population differences in morphology and life history have a genetic component (Price 2008). In addition, numerous studies report significant additive genetic variation of phenotypic traits within populations of passerines (Merilä & Sheldon 2001). For *Zosterops* in particular, significant heritable variation in morphology was detected within the *Z. lateralis* on Heron Island, Australia (Frentiu *et al.* 2007). It is therefore plausible that morphological differences between populations have a large additive genetic component.

The Vanuatu archipelago is conspicuous for its low level of avifaunal endemism compared with neighbouring archipelagos (Mayr & Diamond 2001) and lack of adaptive radiations compared with spectacular examples in honeycreepers from Hawaii (Fleischer & McIntosh 2001) and Darwin's finches on the Galápagos (Grant & Grant 2008). The ancestor of Darwin's finches is believed to have colonized the Galapagos approximately 2.3 Myr ago (Sato *et al.* 2001), thereafter radiating into some 13 species, several of which can now coexist on any single island. In comparison, the ancestor or ancestors of the main *Z. flavifrons* clade are estimated to have colonized Vanuatu sometime in the period 2–4 Myr ago (Phillimore *et al.* 2008*b*; Moyle *et al.* 2009; Black 2010), and has radiated into just two or perhaps three allospecies. The constraining effect of intra-archipelago gene flow is just one of the proposed reasons for the generally low levels of divergence across the archipelago (Diamond & Marshall 1977). Lack of population persistence is also thought to be responsible for limiting avian diversification (Mayr 1965; Diamond & Marshall 1977; Ricklefs & Bermingham 2007), and the higher numbers of endemic species on isolated islands have been attributed to less extinction and turnover (Diamond 1980; Price 2008). In *Zosterops*, the strong phylogenetic and population genetic structure among populations suggest that variation in population persistence is not a key limitation to divergence. However, the extent to which this is true of the Vanuatu avifauna in general remains an open question. Other possible explanations for the low endemism and the absence of adaptive radiations on Vanuatu include close proximity to highly diverse source areas ensuring few niches remain empty (Mayr & Diamond 2001) and lack of strong ecological differences among islands (Mayr 1954). For example, Petren *et al.* (2005) concluded that adaptive divergence among the warbler finches (*Certhidea*) in the Galápagos was limited by stabilizing selection across similar environments. Adaptive radiations may be particularly restricted by the lack of opportunity for sympatry owing to ecological or pathogen incompatibility (Ricklefs & Bermingham 2007). Discriminating which set of conditions place most constraint on avian diversification in Vanuatu requires an accumulation of genetic, morphological and parasite data from numerous species and a much greater understanding of ecology differences among populations.

5. CONCLUSIONS

An influence of migration on population genetic structure appears to persist for very long time periods (up to hundreds or thousands of years) in an archipelago setting. The expected transition to a signature of a drift-mediated system, as island populations become increasingly isolated because of proposed reductions in dispersal ability, was found to occur only partially in an endemic species, despite a long evolutionary history on the archipelago (millions of years). Gene flow dynamics within the colonizing *Zosterops* species were complex, and characterized by a high degree of asymmetrical migration between pairs of populations. No single explanation accounted for these asymmetries; however, a combination of variation in population sizes and population-level dispersal capabilities overlaid with the dominant pattern of north to south migration in the archipelago may all contribute. Among the four populations of *Z. flavifrons* that are likely to exchange migrants, source–sink relationships were also evident. Intra-archipelago phenotypic variation was not obviously influenced by the degree of gene flow or drift experienced by each population. Some weak latitudinal phenotypic patterns found in both species suggested that climate-related variables could partially influence phenotypic divergence via selection; however, numerous other ecological differences among populations await quantification.

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REFERENCES

Adler, G. H., Austin, C. C. & Dudley, R. 1995 Dispersal and speciation of skinks among archipelagos in the tropical Pacific Ocean. *Evol. Ecol.* **9**, 529–541. (doi:10.1007/BF01237834)

Barraclough, T. G. & Vogler, A. P. 2000 Detecting the geographical pattern of speciation from species-level phylogenies. *Am. Nat.* **155**, 419–434. (doi:10.1086/303332)

Berry, R. J. 1998 Evolution of small mammals. In *Evolution on islands* (ed. P. R. Grant), pp. 35–50. Oxford, UK: Oxford University Press.

Black, R. A. 2010 Phylogenetic and phenotypic divergence of an insular radiation of birds. PhD thesis, Imperial College London.

Bollmer, J. L., Whiteman, N. K., Cannon, M. D., Bednarz, J. C., De Vries, T. & Parker, P. G. 2005 Population genetics of the Galapagos hawk (*Buteo galapagoensis*): genetic monomorphism within isolated populations. *Auk* **122**, 1210–1224. (doi:10.1642/0004-8038(2005)122[1210:PGOTGH]2.0.CO;2)

Bolnick, D. I. & Fitzpatrick, B. M. 2007 Sympatric speciation: models and empirical evidence. *Ann. Rev. Ecol. Evol. Syst.* **38**, 459–487. (doi:10.1146/annurev.ecolsys.38.091206.095804)

Bregulla, H. 1992 *Birds of Vanuatu*. Oswestry, UK: Anthony Nelson.

Burney, C. W. & Brumfield, R. T. 2009 Ecology predicts levels of genetic differentiation in neotropical birds. *Am. Nat.* **174**, 358–368. (doi:10.1086/603613)

Butlin, R. K., Galindo, J. & Grahame, J. W. 2008 Sympatric, parapatric or allopatric: the most important way to classify speciation? *Phil. Trans. R. Soc. B* **363**, 2997–3007. (doi:10.1098/rstb.2008.0076)

Ciofi, C., Beaumont, M. A., Swingland, I. R. & Bruford, M. W. 1999 Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. *Proc. R. Soc. Lond. B* **266**, 2269–2274. (doi:10.1098/rspb.1999.0918)

Clegg, S. M., Degnan, S. M., Moritz, C., Estoup, A., Kikkawa, J. & Owens, I. P. F. 2002*a* Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. *Evolution* **56**, 2090–2099.

Clegg, S. M., Degnan, S. M., Kikkawa, J., Moritz, C., Estoup, A. & Owens, I. P. F. 2002*b* Genetic consequences of sequential founding events by an island-colonizing bird. *Proc. Natl Acad. Sci. USA* **99**, 8127–8132. (doi:10.1073/pnas.102583399)

Clegg, S. M., Frentiu, F. D., Kikkawa, J., Tavecchia, G. & Owens, I. P. F. 2008 4000 years of phenotypic divergence in an island bird: heterogeneity in strength of selection over three microevolutionary timescales. *Evolution* **62**, 2393–2410. (doi:10.1111/j.1558-5646.2008.00437.x)

Cody, M. L. & Overton, J. McC. 1996 Short-term evolution of reduced dispersal in island plant populations. *J. Ecol.* **84**, 53–61.

Coyne, J. A. & Orr, H. A. 2004 *Speciation*. MA, USA: Sinauer Associates.

Coyne, J. A. & Price, T. D. 2000 Little evidence for sympatric speciation in island birds. *Evolution* **54**, 2166–2171.

Degnan, S. M., Robertson, B. C., Clegg, S. M. & Moritz, C. 1999 Microsatellite primers for the study of gene flow and mating systems in white-eyes (*Zosterops*). *Mol. Ecol.* **8**, 157–158.

Diamond, J. M. 1980 Species turnover in island bird communities. *Proc. 17th Int. Ornith. Congress, Berlin, Germany, 1978*. Berlin, Germany: Deutsche Ornithologen-Gesellschaft.

Diamond, J. M. & Marshall, A. G. 1977 Distributional ecology of New Hebridean birds: a species kaleidoscope. *J. Anim. Ecol.* **46**, 703–727.

Diamond, J. M., Gilpin, M. E. & Mayr, E. 1976 Species–distance relation for birds of the Solomon Archipelago, and the paradox of the great speciators. *Proc. Natl Acad. Sci. USA* **73**, 2160–2164. (doi:10.1073/pnas.73.6.2160)

Dobzhansky, T. 1937 *Genetics and the origin of species*. New York, NY: Columbia University Press.

Evanno, G., Regnaut, S. & Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)

Faubet, P., Waples, R. S. & Gaggiotti, O. E. 2007 Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Mol. Ecol.* **16**, 1149–1166. (doi:10.1111/j.1365-294X.2007.03218.x)

Falush, D., Stephens, M. & Pritchard, J. K. 2003 Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

Fleischer, R. C. & McIntosh, C. E. 2001 Molecular systematics and biogeography of the Hawaiian avifauna. *Stud. Avian Biol.-Ser.* **22**, 51–60.

Frentiu, F. D., Lange, C. L., Burke, T. & Owens, I. P. F. 2003 Isolation of microsatellite loci in the Capricorn silvereye, *Zosterops lateralis chlorocephalus* (Aves: Zosteropidae). *Mol. Ecol. Notes* **3**, 462–464. (doi:10.1046/j.1471-8286.2003.00484.x)

Frentiu, F. D., Clegg, S. M., Blows, M. W. & Owens, I. P. F. 2007 Large body size in an island dwelling bird: a microevolutionary analysis. *J. Evol. Biol.* **20**, 639–649. (doi:10.1111/j.1420-9101.2006.01242.x)

Goudet, J. 1995 FSTAT version 1.2: a computer program to calculate F statistics. *J. Hered.* **86**, 485–485.

Goudet, J. 2001 FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. See www.unil.ch/izea/softwares/fstat.html.

Grant, P. R. 1998 Patterns on islands and microevolution. In *Evolution on islands* (ed. P. R. Grant), pp. 1–17. Oxford, UK: Oxford University Press.

Grant, P. R. & Grant, B. R. 2008 *How and why species multiply. The radiation of Darwin's finches*. Princeton, NJ: Princeton University Press.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998 A DNA test to sex most birds. *Mol. Ecol.* **7**, 1071–1075. (doi:10.1046/j.1365-294x.1998.00389.x)

Hedrick, P. W. 2005 A standardized genetic differentiation measure. *Evolution* **59**, 1633–1638.

Hinten, G., Harriss, F., Rossetto, M. & Baverstock, P. R. 2003 Genetic variation and island biogeography: microsatellite and mitochondrial DNA variation in island populations of the Australian bush rat, *Rattus fuscipes greyii*. *Conserv. Gen.* **4**, 759–778. (doi:10.1023/B:COGE.0000006113.58749.ac)

Huber, S. K., De León, L. F., Hendry, A. P., Bermingham, E. & Podos, J. 2007 Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proc. R. Soc. B* **274**, 1709–1714. (doi:10.1098/rspb.2007.0224)

Hutchison, D. W. & Templeton, A. R. 1999 Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898–1914. (doi:10.2307/2640449)

Jensen, J. L., Bohonak, A. J. & Kelley, S. T. 2005 Isolation by distance, web service. *BMC Genet.* **6**, 13 v. 3.08. See http://ibdws.sdsu.edu/.

Jordan, M. A. & Snell, H. L. 2008 Historical fragmentation of islands and genetic drift in populations of Galapagos lava lizards (*Microlophus albemarlensis* complex). *Mol. Ecol.* **17**, 1224–1237. (doi:10.1111/j.1365-294X.2007.03658.x)

Jordan, M. A., Snell, H. L., Snell, H. M. & Jordan, W. C. 2005 Phenotypic divergence despite high levels of gene flow in Galapagos lava lizards (*Microlophus albemarlensis*). *Mol. Ecol.* **14**, 859–867. (doi:10.1111/j.1365-294X.2005.02452.x)

Kirchman, J. J. & Franklin, J. D. 2007 Comparative phylogeography and genetic structure of Vanuatu birds: control regions variation in a rail, a dove and a passerine. *Mol. Phylogenet. Evol.* **43**, 14–23. (doi:10.1016/j.ympev.2006.12.013)

Kuehn, R., Schroeder, W., Prichner, F. & Rottmann, O. 2003 Genetic diversity, gene flow and drift in Bavarian red deer populations (*Cervus elaphus*). *Conserv. Gen.* **4**, 157–166. (doi:10.1023/A:1023394707884)

Lack, D. 1971 *Ecological isolation in birds*. Cambridge, MA: Harvard University Press.

Lande, R. & Barrowclough, G. F. 1987 Effective population size, genetic variation, and their use in population management. In *Viable populations for conservation* (ed. M. E. Soule), pp. 87–123. Cambridge, UK: Cambridge University Press.

Leberg, P. L. 2002 Estimating allelic richness: effects of sample size and bottlenecks. *Mol. Ecol.* **11**, 2445–2449. (doi:10.1046/j.1365-294X.2002.01612.x)

Leinonen, T., Cano, J. M., Makinen, H. & Merilä, J. 2006 Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *J. Evol. Biol.* **19**, 1803–1812. (doi:10.1111/j.1420-9101.2006.01182.x)

Lenormand, T. 2002 Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**, 183–189.

Loader, C. 2007 Locfit: local regression, likelihood and density estimation. R package version 1.5–4. See http://cran.r-project.org/web/packages/locfit.

Lopez-Vaamonde, C., Wikström, N., Labandeira, C., Godfray, H. C. J., Goodman, S. J. & Cook, J. M. 2006 Fossil-calibrated molecular phylogenies reveal that leafmining moths radiated millions of years after their host plants. *J. Evol. Biol.* **19**, 1314–1326. (doi:10.1111/j.1420-9101.2005.01070.x)

Mallick, D. I. J. 1975 Development of the New Hebrides archipelago. *Phil. Trans. R. Soc. Lond. B* **275**, 277–285. (doi:10.1098/rstb.1975.0087)

Mayr, E. 1942 *Systematics and the origin of species*. New York, NY: Columbia University Press.

Mayr, E. 1945 *Birds of the southwest Pacific*. New York, NY: Macmillan.

Mayr, E. 1954 Change of genetic environment and evolution. In *Evolution as a process* (ed. E. B. Ford), pp. 157–180. London, UK: Allen & Unwin.

Mayr, E. 1965 Avifauna: turnover on islands. *Science* **150**, 1587–1588. (doi:10.1126/science.150.3703.1587)

Mayr, E. & Diamond, J. 2001 *The birds of northern Melanesia: speciation, ecology and biogeography*. New York, NY: Oxford University Press.

McCall, R. A., Nee, S. & Harvey, P. H. 1998 The role of wing length in the evolution of avian flightlessness. *Evol. Ecol.* **12**, 569–580. (doi:10.1023/A:1006508826501)

McNab, B. H. 1994 Energy conservation and the evolution of flightlessness in birds. *Am. Nat.* **144**, 628–642. (doi:10.1086/285697)

Mees, G. F. 1969 A systematic review of the Indo-Australian Zosteropidae. Part III. *Zool. Verh.* (*Leiden*) **102**, 1–390.

Meirmans, P. G. 2006 Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* **60**, 2399–2402.

Merilä, J. & Sheldon, B. C. 2001 Avian quantitative genetics. *Curr. Ornithol.* **16**, 179–255.

Moyle, R. G., Filardi, C. E., Smith, C. E. & Diamond, J. 2009 Explosive Pleistocene diversification and hemispheric expansion of a 'great speciator'. *Proc. Natl Acad. Sci. USA* **106**, 1863–1868. (doi:10.1073/pnas.0809861105)

Nei, M., Maruyama, T. & Chakraborty, R. 1975 The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10. (doi:10.2307/2407137)

Nicholls, J. A., Double, M. C., Rowell, D. M. & Magrath, D. 2000 The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). *J. Avian Biol.* **31**, 165–176. (doi:10.1034/j.1600-048X.2000.310208.x)

Nosil, P. 2008 Speciation with gene flow could be common. *Mol. Ecol.* **17**, 2103–2106. (doi:10.1111/j.1365-294X.2008.03715.x)

Ogden, R. & Thorpe, R. S. 2002 Molecular evidence for ecological speciation in tropical habitats. *Proc. Natl Acad. Sci. USA* **99**, 13 612–13 615. (doi:10.1073/pnas.212248499)

Petren, K. 1998 Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. *Mol. Ecol.* **7**, 1782–1784.

Petren, K., Grant, B. R. & Grant, P. R. 1999 A phylogeny of Darwin's finches based on microsatellite DNA length variation. *Proc. R. Soc. Lond. B* **266**, 321–329. (doi:10.1098/rspb.1999.0641)

Petren, K., Grant, P. R., Grant, B. R. & Keller, L. F. 2005 Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Mol. Ecol.* **14**, 2843–2957.

Phillimore, A. B. 2006 The ecological basis of speciation and divergence in birds. PhD thesis, Imperial College London.

Phillimore, A. B., Orme, C. D. L., Thomas, G. H., Blackburn, T. M., Bennett, P. M., Gaston, K. J. & Owens, I. P. F. 2008*a* Sympatric speciation in birds is rare: insights from range data and simulations. *Am. Nat.* **171**, 646–657. (doi:10.1086/587074)

Phillimore, A. B., Owens, I. P. F., Black, R. A., Chittock, J., Burke, T. & Clegg, S. M. 2008*b* Complex patterns of genetic and phenotypic divergence in an island bird and the consequences for delimiting conservation units. *Mol. Ecol.* **17**, 2839–2853. (doi:10.1111/j.1365-294X.2008.03794.x)

Price, T. D. 2008 *Speciation in birds*. Greenwood Village, CO: Roberts.

Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

R Development Core Team 2008 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raymond, M. & Rousset, F. 1995 GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2226–2234.

Ricklefs, R. E. & Bermingham, E. 1999 Taxon cycles in the Lesser Antillean avifauna. *Ostrich* **70**, 49–59.

Ricklefs, R. E. & Bermingham, E. 2007 The causes of evolutionary radiations in archipelagos: passerine birds of the Lesser Antilles. *Am. Nat.* **169**, 285–297. (doi:10.1086/510730)

Rousset, F. 1997 Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**, 1219–1228.

Rundell, R. J. & Price, T. D. 2009 Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* **24**, 394–399. (doi:10.1016/j.tree.2009.02.007)

Rundle, H. D. & Nosil, P. 2005 Ecological speciation. *Ecol. Lett.* **8**, 336–352. (doi:10.1111/j.1461-0248.2004.00715.x)

Ryan, P. G., Bloomer, P., Moloney, C. L., Grant, T. J. & Delport, W. 2007 Ecological speciation in south Atlantic

island finches. *Science* **315**, 1420–1423. (doi:10.1126/science.1138829)

Sato, A., Tichy, H., O'hUigin, C., Grant, P. R., Grant, B. R. & Klein, J. 2001 On the origin of Darwin's finches. *Mol. Biol. Evol.* **18**, 299–311.

Schluter, D. 2009 Evidence for ecological speciation and its alternative. *Science* **323**, 737–741. (doi:10.1126/science.1160006)

Skjelseth, S., Ringsby, T. H., Tufto, J., Jensen, H. & Saether, B.-E. 2007 Dispersal of introduced house sparrows *Passer domesticus*: an experiment. *Proc. R. Soc. B* **274**, 1763–1771. (doi:10.1098/rspb.2007.0338)

Slatkin, M. 1987 Gene flow and the geographic structure of natural populations. *Science* **236**, 787–792. (doi:10.1126/science.3576198)

Slatkin, M. 1993 Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264–279. (doi:10.2307/2410134)

Smith, C. E. & Filardi, C. E. 2007 Patterns of molecular and morphological variation in some Solomon Island land birds. *Auk* **124**, 479–493. (doi:10.1642/0004-8038(2007)124[479:POMAMV]2.0.CO;2)

Smith, A. L. & Friesen, V. L. 2007 Differentiation of sympatric populations of the band-rumped storm-petrel in the Galapagos Islands: an examination of genetics, morphology, and vocalizations. *Mol. Ecol.* **16**, 1593–1603. (doi:10.1111/j.1365-294X.2006.03154.x)

Sorenson, M. D., Sefc, K. M. & Payne, R. B. 2003 Speciation by host switch in brood parasitic indigobirds. *Nature* **424**, 928–931. (doi:10.1038/nature01863)

Spitze, K. 1993 Population-structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* **135**, 367–374.

Wilson, G. A. & Rannala, B. 2003 Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177–1191.