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Genetic differentiation within a widespread "supertramp" taxon: Molecular phylogenetics of the Louisiade White-eye (*Zosterops griseotinctus*) ☆

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ABSTRACT

"Supertramp" species are highly specialized overwater dispersers, and are useful taxa for investigating the influence of dispersal ability on speciation and diversification in island settings. The Louisiade White-eye (*Zosterops griseotinctus*) is a widespread avian supertramp endemic to Papua New Guinea's offshore islands. We used maximum likelihood and Bayesian inference to reconstruct phylogenetic relationships based on 2 mitochondrial and 1 nuclear loci (1813 bp total) from 88 individuals representing all 4 named subspecies and the full breadth of the species' range. We found significant geographic and population genetic structure, and support for a major clade containing the coral islets of the central Louisiade Archipelago and outlying Nissan Island. We found evidence of metapopulation structure and gene flow within the Louisiade Archipelago clade, and relatively high genetic distinctiveness of outlying island populations, including the population on volcanically-defaunated Long Island. We reject a hypothesis of panmixia within the Louisiade White-eye despite their long-range dispersal ability, and find evidence of selection against dispersal ability in populations on high-elevation islands where disturbance is rare. Our study represents a rare intraspecies phylogeny of an avian supertramp, and sheds light on patterns of evolution in highly vagile island species.

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1. Introduction

Variation in dispersal ability across groups of organisms is a critical factor in the genesis of biological diversity and novel species (Diamond and Mayr, 2001). New species arise when barriers to gene flow emerge between taxa (Dobzhansky, 1937; Mayr, 1942). Barriers to gene flow can be geographic or behavioral, or arise through adaptive evolution in response to varied ecological conditions. Geographic barriers physically restrict the transfer of alleles among populations, as in vicariance and allopatric speciation (Wright, 1931; Mayr, 1942). In sympatric populations, reproductive isolation can arise through behavioral barriers such as species recognition (Shaw and Parsons, 2002; Seehausen et al., 2008; Uy et al., 2009), or from divergence driven by varied selective pressures across an environmental gradient, as in ecological speciation (Rundle and Nosil, 2005; Nosil, 2012). As dispersal ability influences relative rates of gene flow, it can determine whether these barriers and selective pressures are sufficiently strong to result in reproductive isolation.

Dispersal ability is particularly significant in island contexts, where the evolutionary trajectories of organisms are strongly influenced by their ability to cross open water. Highly sedentary species rarely reach other islands, and once there, in the absence of gene flow may rapidly diverge. Highly vagile species may frequently cross between islands, maintaining gene flow and precluding divergence. While groups of sedentary species in island contexts populate the literature as classic examples of adaptive radiations (Lovette et al., 2002; Baldwin and Robichaux, 1995; Grant, 1981), the evolutionary histories of highly vagile species remain poorly understood (but see Andersen et al., 2014).

One group of highly vagile species with shared ecological and distributional characteristics have been termed supertramps (*sensu* Diamond, 1974). Avian supertramps are prevalent in the island

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avifaunas of Melanesia in the southwest Pacific, and are characterized by their occurrence primarily on small islands with low alpha diversity (Diamond and Mayr, 2001; Andersen et al., 2014; Jønsson et al., 2008). Based on these characteristics and field observations, Diamond hypothesizes supertramps represent an extreme case of *r*-selection in birds: they are highly specialized for rapid breeding and overwater dispersal, but at the expense of competitive ability and refined local adaptation (Diamond, 1974). He posits that as a result, they are restricted in distribution to islets with relatively marginal habitat and few competitors, and to islands defaunated by volcanic eruptions with lower species numbers than equilibrium expectations (Diamond, 1974).

Clades that include both supertramps and less vagile species are useful taxa for investigating the influence of lineage-specific life-history traits on rates of speciation (Moyle et al., 2009). Further, they can help address a famous paradox: how can lineages show such high degrees of differentiation across oceanic islands when their necessarily excellent dispersal ability and resulting gene flow should limit divergence? Moyle et al. (2009) explored this question by studying the pattern and tempo of speciation in the Zosteropidae (White-eyes), a species-rich avian family with high rates of endemism and a concentration in the tropical Pacific and Indian Oceans. They reconstructed phylogenetic relationships in the *Zosterops* [*griseotinctus*] superspecies, a Melanesian species group containing multiple highly sedentary taxa and a single widespread supertramp. Concluding that a rapid shift in dispersal ability may have played a prominent role in the diversification of White-eyes, their study suggests that disparate populations within a supertramp species may represent incipient species. Their results highlight the potential for intraspecific studies of supertramps to shed light on the balance of natural processes underpinning speciation.

The nominate race of the *Zosterops* [*griseotinctus*] group, the Louisiade White-eye, *Z. griseotinctus*, is a supertramp species inhabiting islands in the Louisiade, Bismarck, and Admiralty archipelagos of Papua New Guinea. Current taxonomy recognizes four named subspecies spread across the species' 800 linear mile range, distinguished by their disjunct ranges and by minor differences in bill length (van Balen, 2008; Pratt and Beehler, 2014). These are: *Z. g. griseotinctus* in the western Louisiades, *Z. g. longirostris* in the eastern Louisiades, *Z. g. pallidipes* on Rossel Island, and *Z. g. eichhorni* on the islands of the Vitiaz Strait, Nissan Island, and Admiralty Archipelago (Fig. 1; van Balen, 2008). Additional available names include Admiralty group populations as *Z. g. ottomeyeri* and Misima Island population as *Z. g. aignani* (van Balen, 2008; ITIS, 2014). For consistency, we follow van Balen (2008) in synonymizing *Z. g. ottomeyeri* with *Z. g. eichhorni*, and *Z. g. aignani* with the nominate subspecies.

Similar to other Melanesian supertramps, *Z. gristeotinctus* is primarily restricted in distribution to small coral islets, such as those comprising the central Louisiade Archipelago. However, *Z. griseotinctus* is also found on several relatively large, mountainous (hereafter "high") islands across its range, where it occurs in both typical littoral habitat as well as primary lowland and montane moist forest: Rossel, Long, Crown, and Tolokiwa Islands. The latter three islands are particularly interesting in having suffered volcanic defaunation during Long Island's most recent violent eruption in the late 1600s, and are held as evidence of the species' dispersal ability and limited tolerance for interspecies competition (Diamond, 1974). Despite the geographic and ecological breadth of its distribution, no field or molecular studies to date have investigated levels of gene flow among populations of *Z. griseotinctus*.

Here, we present a phylogenetic hypothesis for the Louisiade White-eye, *Zosterops griseotinctus*. We sample three loci (two mitochondrial and one nuclear) from 88 individuals representing all four named subspecies across the species' range, and reconstruct phylogenetic relationships using Bayesian inference. We test for a genetic signal consistent with the supertramp hypothesis, with an expectation of genetic homogeneity. We also use phylogeographic patterns among sampled populations to investigate two

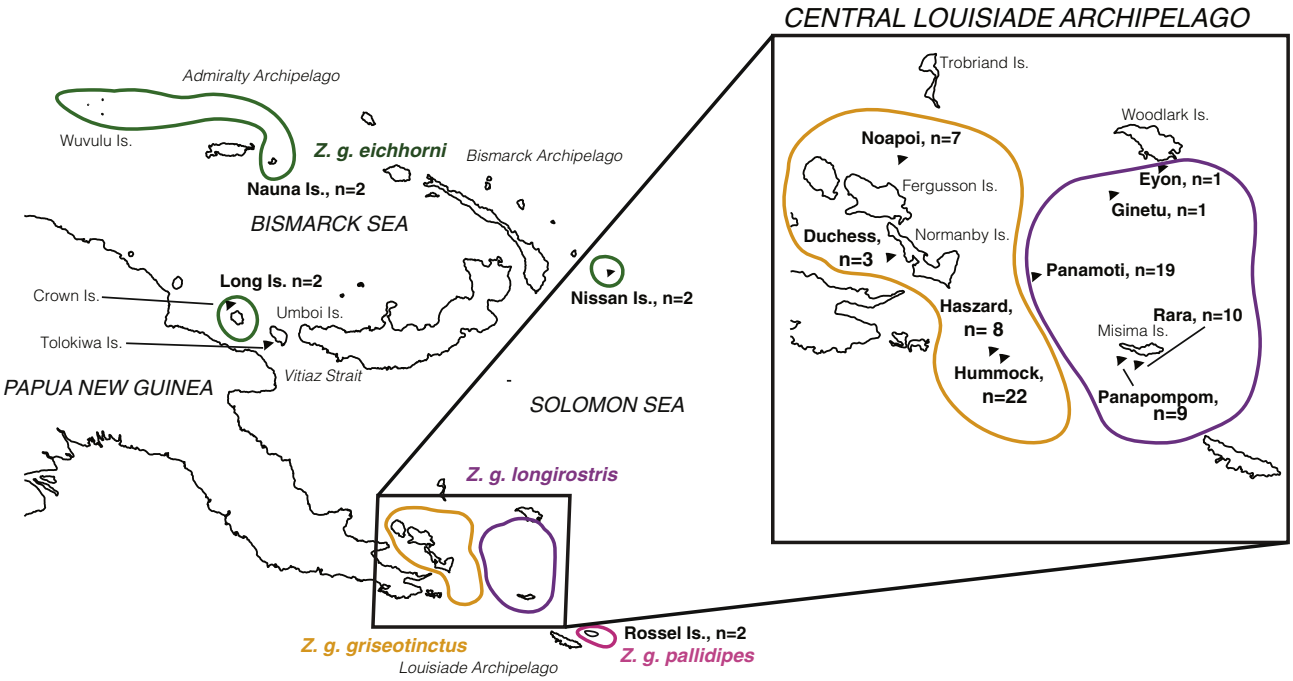

Fig. 1. Sampling locations for *Z. griseotinctus*. Boldface island names denote sampling localities, and are annotated with sample size. Known unsampled populations are marked by plain text island names within illustrated range limits. Subspecies ranges are color coded, with green indicating *Z. g. eichhorni*, pink indicating *Z. g. pallidipes*, orange indicating *Z. g. griseotinctus*, and purple indicating *Z. g. longirostris*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

alternate hypotheses about the evolutionary history of supertramp species. If supertramp status is temporary and disparate populations represent incipient species, we expect varied levels of genetic divergence among populations. Alternatively, if supertramp status is maintained through consistent dispersal, we expect relatively uniform genetic divergence among populations.

2. Methods

2.1. Taxon sampling and DNA sequencing

We sampled tissues from 88 individuals within *Zosterops griseotinctus*, as well as three outgroup species: *Z. atrifrons*, *Z. japonicus*, and *Z. pallidus*. Our taxonomic sampling includes all four named subspecies of *Z. griseotinctus*, and represents the full breadth of the species' geographical range, with samples from 14 distinct islands (Fig. 1; Table 1). Our sampling also includes populations historically considered taxonomically distinct but now lumped under another subspecific epithet (see above). Sampling was conducted by the authors, and augmented by pre-existing museum samples of outlying populations. Our sampling was designed to represent the greatest breadth of geologic regions and island types, and focused on populations on the atolls of the central Louisiade Archipelago ($n = 80$ individuals over 9 islands). Here, in the geographic center of diversity and location of the bulk of known populations (Tarburton, 2015), we attempted to visit each major island group from the area roughly bounded by the D'Entrecasteaux Islands, the Trobriand Islands, Budibudi Atoll, and the Louisiade Islands (excluding better-studied Rossel and Sudest). Among the clusters of islands within this area, we attempted to sample one larger, human-inhabited island, and one uninhabited coral islet. Sampling covered the most geographically distant known populations in this region, and confirmed a range extension for the Amphlett Group (represented here by Normanby and Duchess Islands). Table 1 includes key data from all individuals included in the study. Our fieldwork also confirmed that *Z. griseotinctus* occurs widely primarily on smaller atolls and islands with low alpha diversity.

From fresh tissues, DNA was isolated using a Qiagen DNeasy kit following the manufacturer's recommended protocol. When fresh tissues were not available, DNA was extracted from the toe pads of museum study skins in a dedicated ancient DNA laboratory at the California Academy of Sciences using a phenol–chloroform and centrifugal dialysis method (Dumbacher and Fleischer, 2001). No modern DNA or post-polymerase chain reaction products were handled in this lab, located on a separate floor from the main genetics facility, and controls for detecting contamination were implemented.

Polymerase chain reaction (PCR) was used to amplify two regions of the mitochondrial DNA and one nuclear intron (Table 2). Mitochondrial loci were ATP8+, a 313 bp region comprised of 158 bp from ATP8, 70 bp of t-lysine, and 85 bp at the 3′ end of the COII gene, and NADH2, approximately 900 bp. The nuclear intron was TGFB2, and approximately 600 bp. Due to the degraded nature of DNA from museum skins, we could only amplify DNA in small segments of DNA from our targeted regions (~150–200 bp), which were later concatenated. PCRs utilized AmpliTaq Gold® DNA Polymerase (Life Technologies brand, ThermoFisher Scientific Inc., Grand Island, NY) and standard components and cycling profiles, with an initial 10 min denaturation at 95 °C before thermocycling up to 45 cycles at the following profile: 30 s at 94 °C, 15 s of annealing at 50 °C, 1 min of extension at 72 °C, and a final extension of 5 min at 72 °C. Success of PCR was assessed by gel electrophoresis, and products were purified. Forward and reverse DNA strands were sequenced using Life Technologies BigDye® Terminator v3.1 Cycle Sequencing Kit reactions run on an Life Technology 3130xL Genetic Analyzer capillary sequencer. We edited and manually assembled forward and reverse sequences for each individual and each locus using Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI).

2.2. Phylogenetic analyses

We determined the best-fit models of evolution for each locus with jModeltest v2.1.4. (Posada, 2008) using Bayesian Information Criterion (BIC; Burnham and Anderson, 2003). We used a Bayes factor comparison implemented in MrBayes 3.2.1 to test for clock-like rates using both the rough harmonic mean and stepping stone models (Ronquist et al., 2012). We then estimated phylogenetic trees with the concatenated mtDNA dataset, using a strict Bayesian molecular clock framework implemented in BEAST v1.8.1 (Drummond et al., 2012). We partitioned each locus, and set both independent models and rates of nucleotide evolution. Rates values were set at 0.029 and 0.019 for ND2 and ATP8+, respectively, and represent the number of substitutions per site per million years, derived from estimates produced for use in avian phylogenetic studies from Lerner et al. (2011). We conducted BEAST analyses for 40 million MCMC generations, with samples retained every 1000 generations, using Coalescent constant size tree priors for the mtDNA dataset. We displayed results in TRACER v1.5 (Rambaut and Drummond, 2007) to confirm acceptable mixing and likelihood stationarity, appropriate burn-in, and adequate effective sample sizes (ESS) above 200 for all estimated parameters. After discarding the first 10 million generations (25%) as burn-in, we summarized the parameter values of the samples from the posterior distribution on the maximum clade credibility tree using TreeAnnotator v1.8.1 (Drummond et al., 2012). We also conducted maximum likelihood analyses separately on both the concatenated mtDNA dataset and the nuclear locus dataset using RAxML v7.2.6 (Stamatakis, 2006) under the recommended GTRGAMMA + I model, and used 1000 nonparametric rapid bootstrap replicates to assess nodal support. For the nuclear locus dataset, we produced a median-joining haplotype network with PopART 1.5 (http://popart.otago.ac.nz.), masking ambiguous sites and gaps in our alignment.

We used linear regressions of uncorrected genetic distance between samples to test three island biogeographic models. In each model, we examined the correlation of genetic distance between nearest neighbor populations with (A) island area, (B) island elevation, or (C) interisland distance. Model A tests whether adjacent allopatric populations are more or less likely to diverge based on home island size, with an expectation of a positive correlation between divergence and island area. Model B tests whether adjacent allopatric populations are more or less likely to diverge based on home island elevation, with an expectation of a positive correlation between divergence and maximum elevation. Model C tests whether the level of divergence between adjacent allopatric populations is related to the distance between populations. Thus, models A and B test for reduced dispersal in supertramp populations on larger, less disturbance-prone islands, while model C tests a classic isolation by distance hypothesis.

3. Results

We obtained full-length DNA sequences from all frozen tissue and shorter sequences from toe pads. Our matrix contained 91 samples from 4 taxa and 1813 base pairs from three sequenced amplicons: ND2, ATP8+, and TGFB2. The concatenated mtDNA alignment contained 58 parsimony-informative sites (ND2: 46, ATP8+; 12), and showed much greater variability than the nuclear locus TGFB2 (4 parsimony informative sites out of 601 sites). The

Table 1
List of samples used in the study following the taxonomy of van Balen (2008). Ancient DNA samples derived from museum specimens denoted (*). Museum catalog numbers are provided for vouchered specimens, and field numbers are provided for tissues that do not have voucher specimens. GenBank accession numbers are provided for each sample at each locus.

Museum catalog or field number	Subspecies	Locality	ND2	ATP8+	TGFB2
AMNH329017*	*Z. g. eichorni*	Nissan	KT310488	KT310577	KT310678
AMNH329018*	*Z. g. eichorni*	Nissan	KT310493	KT310578	KT310674
AMNH330113*	*Z. g. pallipides*	Rossel	KT310494	KT310579	KT310672
AMNH330116*	*Z. g. pallipides*	Rossel	KT310492	KT310580	KT310675
AMNH335071*	*Z. g. ottomeyeri*	Nauna	KT310491	KT310584	N/A
AMNH335072*	*Z. g. ottomeyeri*	Nauna	KT310490	KT310581	KT310673
AMNH422684*	*Z. g. sp.*	Long Island	KT310487	KT310583	KT310677
AMNH422685*	*Z. g. sp.*	Long Island	KT310489	KT310582	KT310676
DPM502	*Z. g. griseotinctus*	Hummock	KT310471	KT310546	KT310655, KT310656
DPM503	*Z. g. griseotinctus*	Hummock	KT310462	KT310545	KT310669, KT310670
DPM508	*Z. g. griseotinctus*	Hummock	KT310472	KT310544	KT310603
DPM509	*Z. g. griseotinctus*	Hummock	KT310460	KT310543	KT310637
DPM510	*Z. g. griseotinctus*	Hummock	KT310464	KT310542	KT310645
DPM511	*Z. g. griseotinctus*	Hummock	KT310473	KT310541	KT310625
DPM515	*Z. g. griseotinctus*	Hummock	KT310486	KT310540	KT310605
DPM517	*Z. g. griseotinctus*	Hummock	KT310467	KT310500	KT310648
DPM519	*Z. g. griseotinctus*	Haszard	KT310469	KT310498	KT310650
DPM521	*Z. g. griseotinctus*	Haszard	KT310475	KT310539	N/A
DPM523	*Z. g. griseotinctus*	Haszard	KT310479	KT310499	KT310621
DPM531	*Z. g. longirostris*	Panapompom	KT310483	KT310537	KT310633
DPM532	*Z. g. longirostris*	Panapompom	KT310448	KT310538	KT310663
DPM538	*Z. g. longirostris*	Panapompom	KT310456	KT310562	KT310629
DPM540	*Z. g. longirostris*	Rara	KT310465	KT310563	KT310658
DPM543	*Z. g. longirostris*	Rara	KT310466	KT310564	KT310666, KT310667
DPM545	*Z. g. longirostris*	Rara	KT310474	KT310565	KT310634
DPM548	*Z. g. longirostris*	Rara	KT310482	KT310569	KT310664, KT310665
DPM549	*Z. g. longirostris*	Rara	KT310446	KT310568	KT310631
DPM553	*Z. g. longirostris*	Rara	KT310443	KT310567	KT310612, KT310613
DPM634	*Z. g. griseotinctus*	Noapoi	KT310450	KT310566	KT310617
DPM635	*Z. g. griseotinctus*	Noapoi	KT310444	KT310560	KT310668
DPM636	*Z. g. griseotinctus*	Noapoi	KT310445	KT310561	KT310610
DPM637	*Z. g. griseotinctus*	Noapoi	KT310447	KT310559	KT310638
GLGS2246	*Z. japonicus*	China	KT310496	KT310585	KT310679
JF3014	*Z. atrifrons*	Normanby	KT310497	KT310587	KT310681, KT310682
CAS97869	*Z. g. griseotinctus*	Duchess	KT310410	KT310576	KT310599
CAS97871	*Z. g. griseotinctus*	Duchess	KT310421	KT310556	KT310630
CAS97881	*Z. g. griseotinctus*	Noapoi	KT310409	KT310555	N/A
JF3056	*Z. g. longirostris*	Panamoti	KT310484	KT310511	KT310607
JF3058	*Z. g. longirostris*	Panamoti	KT310407	KT310554	KT310614
JF3065	*Z. g. longirostris*	Panamoti	KT310422	KT310571	KT310627
JF3069	*Z. g. longirostris*	Panamoti	KT310485	KT310513	KT310594
CAS97899	*Z. g. longirostris*	Ginetu	KT310459	KT310514	KT310652
CAS96806	*Z. g. griseotinctus*	Hummock	KT310423	KT310515	KT310609
JPD437	*Z. g. griseotinctus*	Hummock	KT310426	KT310519	KT310636
JPD438	*Z. g. griseotinctus*	Hummock	KT310420	KT310574	KT310649
JPD442	*Z. g. griseotinctus*	Hummock	KT310411	KT310517	KT310643
JPD444	*Z. g. griseotinctus*	Hummock	KT310425	KT310516	KT310628
JPD451	*Z. g. griseotinctus*	Hummock	KT310424	KT310510	KT310654
JPD452	*Z. g. griseotinctus*	Hummock	KT310480	KT310509	KT310639
JPD455	*Z. g. griseotinctus*	Hummock	KT310412	KT310508	KT310615, KT310618
JPD457	*Z. g. griseotinctus*	Hummock	KT310413	KT310507	N/A
JPD462	*Z. g. griseotinctus*	Hummock	KT310478	N/A	KT310671
JPD465	*Z. g. griseotinctus*	Hummock	KT310414	KT310506	KT310620
JPD466	*Z. g. griseotinctus*	Hummock	KT310419	KT310505	KT310657
JPD468	*Z. g. griseotinctus*	Hummock	KT310418	KT310575	KT310623
JPD473	*Z. g. griseotinctus*	Haszard	KT310415	KT310503	KT310626
JPD475	*Z. g. griseotinctus*	Haszard	KT310417	KT310502	KT310592
JPD480	*Z. g. griseotinctus*	Haszard	KT310416	KT310501	N/A
JPD481	*Z. g. griseotinctus*	Haszard	KT310435	KT310529	KT310659
CAS96809	*Z. g. longirostris*	Panapompom	KT310437	KT310531	KT310596, KT310597
JPD487	*Z. g. longirostris*	Panapompom	KT310437	KT310533	KT310608
JPD491	*Z. g. longirostris*	Panapompom	KT310438	KT310536	KT310661
CAS96810	*Z. g. longirostris*	Panapompom	KT310442	KT310535	KT310604
JPD500	*Z. g. longirostris*	Panapompom	KT310442	KT310534	KT310632
JPD502	*Z. g. longirostris*	Panapompom	KT310470	KT310528	KT310611
JPD509	*Z. g. longirostris*	Rara	KT310461	KT310527	KT310653
CAS96808	*Z. g. longirostris*	Rara	KT310458	KT310526	KT310616
JPD512	*Z. g. longirostris*	Rara	KT310468	KT310525	KT310593
JPD517	*Z. g. longirostris*	Rara	KT310463	KT310524	KT310588
JPD780	*Z. g. griseotinctus*	Duchess	KT310441	KT310523	KT310640
JPD805	*Z. g. griseotinctus*	Noapoi	KT310440	KT310522	KT310619
JPD822	*Z. g. longirostris*	Panamoti	KT310449	KT310521	KT310644

Table 1 (*continued*)

Museum catalog or field number	Subspecies	Locality	ND2	ATP8+	TGFB2
JPD823	*Z. g. longirostris*	Panamoti	KT310434	KT310520	KT310635
JPD827	*Z. g. longirostris*	Panamoti	KT310433	KT310532	KT310651
JPD828	*Z. g. longirostris*	Panamoti	KT310432	KT310573	KT310606
JPD830	*Z. g. longirostris*	Panamoti	KT310454	KT310530	KT310589
JPD831	*Z. g. longirostris*	Panamoti	KT310431	KT310570	KT310598
JPD846	*Z. g. longirostris*	Eyon	KT310453	KT310512	KT310593
CAS97937	*Z. g. longirostris*	Panamoti	KT310481	KT310558	KT310641, KT310642
MACD10	*Z. g. longirostris*	Panamoti	KT310477	KT310527	KT310662
MACD11	*Z. g. longirostris*	Panamoti	KT310476	KT310557	KT310600
MACD12	*Z. g. longirostris*	Panamoti	KT310428	KT310557	KT310622
MACD3	*Z. g. longirostris*	Panamoti	KT310430	KT310551	KT310595
MACD5	*Z. g. longirostris*	Panamoti	KT310429	KT310504	KT310624
MGD26	*Z. pallidus*	South Africa	KT310495	KT310586	KT310680
TMB051	*Z. g. griseotinctus*	Hummock	KT310457	KT310553	KT310646, KT310647
CAS96811	*Z. g. griseotinctus*	Haszard	KT310455	KT310572	N/A
CAS97961	*Z. g. griseotinctus*	Noapoi	KT310452	KT310550	KT310660
CAS97968	*Z. g. longirostris*	Panamoti	KT310451	KT310549	KT310591
ZRH871	*Z. g. longirostris*	Panamoti	KT310427	KT310548	KT310590
ZRH873	*Z. g. longirostris*	Panamoti	KT310408	KT310547	KT310601

Table 2
Newly designed and pre-existing primers used in the study.

Primer name	Primer sequence	Region	Direction	Citation
TGFB2-5	GAAGCGTGCTCTAGATGCTG	TGF-B	Forward	Moyle et al. (2009)
TGFB2-6	AGGCAGCAATTATCCTGCAC	TGF-B	Reverse	Moyle et al. (2009)
zg.oldND2.F	CGCCTAAGGCTGTTGAGAGT	ND2	Forward	Developed by E.B.L
zg.oldND2.R	ACATCAGCCATCGCAATAAA	ND2	Reverse	Developed by E.B.L
5215-ND2	TATCGGGCCCATACCCCGAAAAT	ND2	Forward	Slikas et al. (2000)
6113-ND2	CAGTATGCAAGTCGGAGGTAGAAG	ND2	Reverse	Slikas et al. (2000)
ND2-Zg-internalForward	AAAACTCCCACCAATTACCC	ND2	Forward	Developed by J.P.D.
ND2-Zg-internalReverse	AGGTGGGAGATGGAGGAGAA	ND2	Reverse	Developed by J.P.D.
CO2GQL	GGACAATGCTCAGAAATCTGCGG	ATPase8	Forward	Slikas et al. (2000)
BirdsRUS	TGGTCGAAGAAGCTTAGGTTC	ATPase8	Reverse	Slikas et al. (2000)
t-Lys	CACCAGCACTAGCCTTTTAAG	ATPase8	Forward	Deiner et al. (2009)

concatenated mtDNA alignment collapsed our 88 *Z. griseotinctus* sequences into 29 unique haplotypes, while the TGFB2 alignment collapsed all sequences into 6 unique alleles. Intraspecific uncorrected genetic distance (*p*-distances) ranged from 0 to 0.03923, and interspecific uncorrected genetic distance ranged from 0.04837 to 0.08283 (Table S1).

Using BIC, the program jModeltest chose an HKY model of nucleotide substitution for ATP8+ and an HKY + G model for ND2. Our analysis of the full concatenated mtDNA matrix in BEAST resulted in ESS values >200 for all parameters, including posterior, prior, and likelihood. No strongly supported topological conflicts occurred between the Bayesian (Fig. 2) and maximum likelihood mtDNAtrees (Fig. S1). In the full concatenated mtDNA tree, both BEAST and RAxML recovered a highly supported (posterior probability = 100; bootstrap = 96) clade containing all *Z. griseotinctus* samples. Maximum likelihood analysis of TGFB2 proved uninformative, producing a weakly supported polytomy of all *Z. griseotinctus* haplotypes. The median-joining haplotype network of our TGFB2 alignment showed 4 weakly structured haplotypes present among all samples (Fig. 3).

Models testing genetic distance between nearest neighbors and island area (A), island height (B) and interisland distance (C) showed statistically significant positive correlations and relatively good fits ($p < 0.02$ and $R^2 = 0.44$ for Model A; $p < 0.04$ and $R^2 = 0.355$ for Model B; $p < 0.03$ and $R^2 = 0.394$ for Model C). These results offer support for all three of the island biogeographic hypotheses we investigated: adjacent allopatric populations appear more likely to diverge when home islands are larger, more likely to diverge when home islands are higher, and more likely to diverge when interisland distance is greater.

4. Discussion

4.1. Phylogenetics and phylogeography

Our study confirms the monophyly of *Z. griseotinctus* across its range relative to our outgroup, Black-crowned White-eye *Z. atrifrons*. However, within *Z. griseotinctus*, we observe significant genetic divergence (up to 3.9% uncorrected *p* distance) and phylogeographic structure. We interpret these data as failing to support a hypothesis of genetic homogeneity (panmixia) in supertramps: even within taxa exhibiting classic supertramp traits and little morphological variation, the rate of gene flow can vary substantially. This result also offers support for the conclusion in Moyle et al. (2009) that rapid shifts in dispersal ability may explain the 'famous paradox' of differentiation in widespread oceanic taxa. Within *Z. griseotinctus*, we see evidence of differentiation that could reflect similar shifts in dispersal ability, and eventually lead to speciation.

Within *Z. griseotinctus*, we find a geographic basis for major clades and populations. In our mitochondrial DNA phylogeny, we recovered a large, well-supported clade containing all samples from the Louisiade Archipelago (subspecies *Z. g. griseotinctus* and *Z. g. longirostris*) and the island of Nissan (subspecies *Z. g. eichhorni*) (Fig. 2, node A). The inclusion of the Louisiade samples in a single clade was expected given the small scale and relative proximity of the Louisiade Islands. Nissan's inclusion in this clade is somewhat less intuitive. While geographically closer to the Louisiade archipelago than the "outlying" islands in the Admiralty group and Vitiaz Strait, Nissan is nonetheless farther away than immediately adjacent populations on the island of Rossel (subspecies *Z. g. pallidipes*).

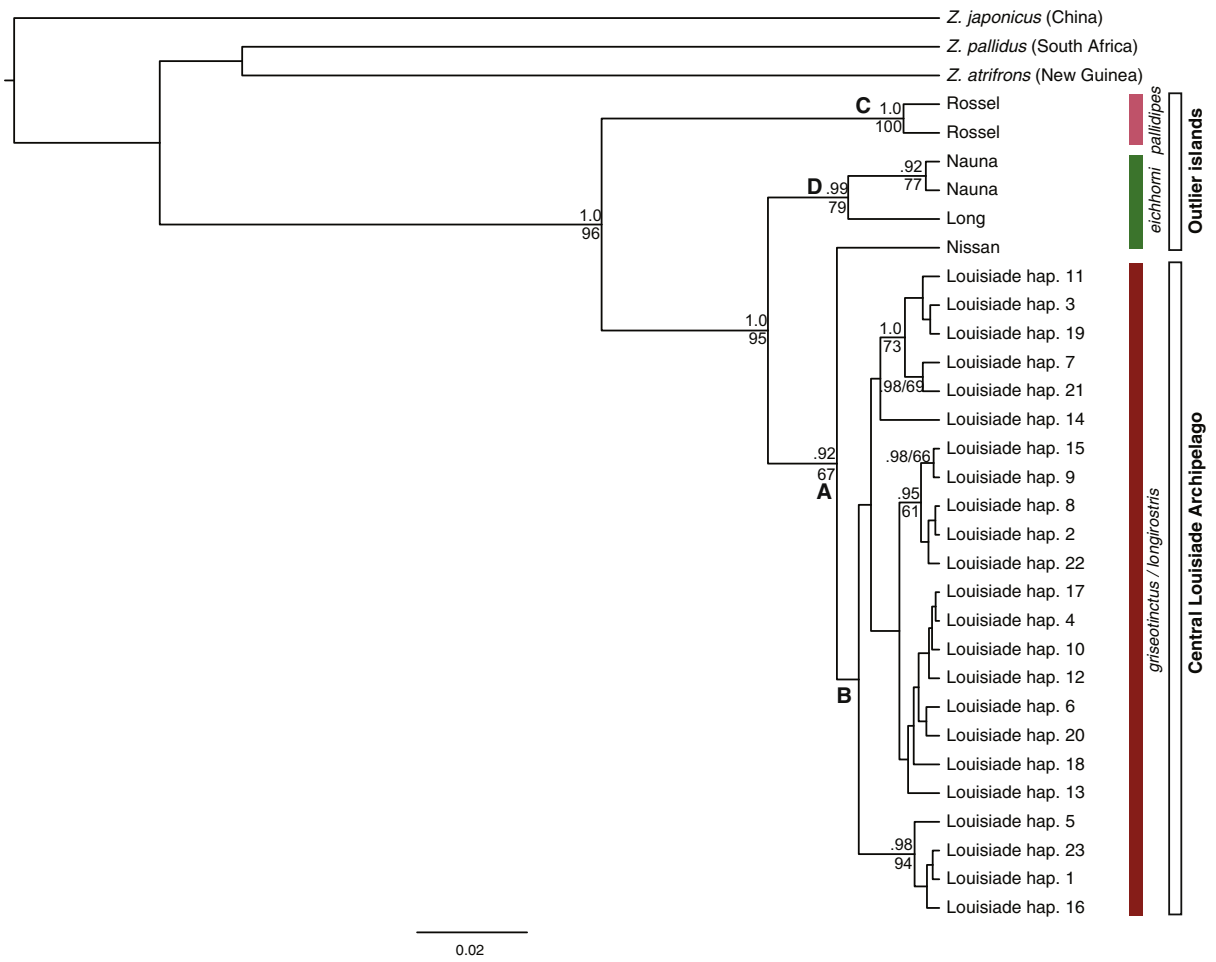

Fig. 2. Phylogenetic relationships among *Zosterops griseotinctus* populations reconstructed with BEAST 1.8.1 from mitochondrial DNA. Numbers above branches indicate Bayesian posterior probabilities for the adjacent node, while numbers below branches indicate maximum likelihood bootstrap support values. Unlabeled nodes received posterior probability values <0.75. Letters adjacent to major nodes are referenced in the text. Central Louisiade Archipelago haplotypes contain samples from both subspecies *Z. g. griseotinctus* and *Z. g. longirostris*.

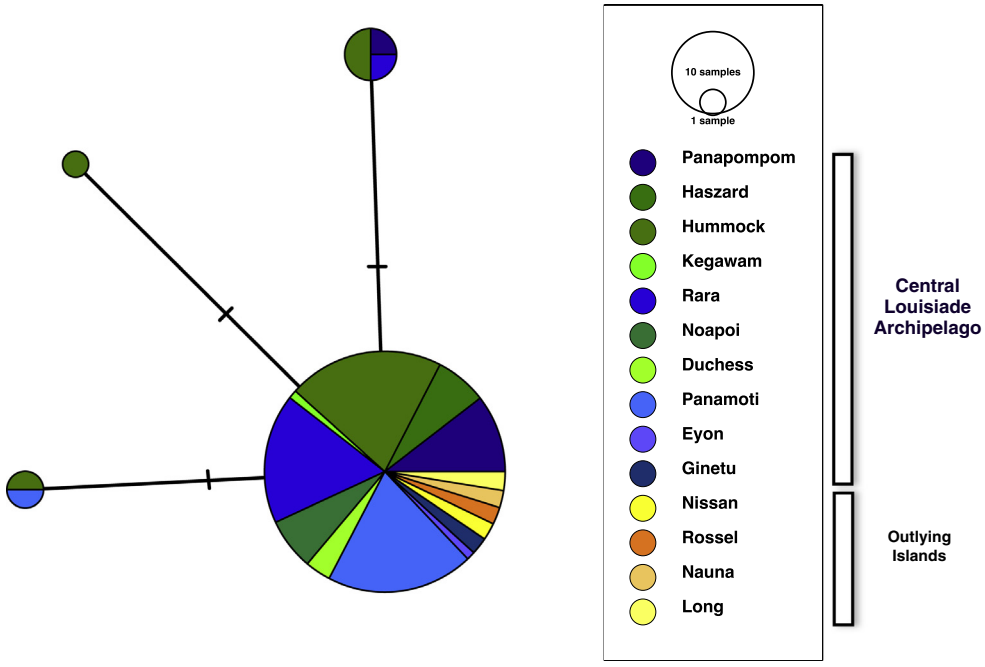

Fig. 3. Relationships of nuclear DNA alleles from samples across the range of *Z. griseotinctus:* median-joining haplotype network estimated from phased alleles of nuclear locus TGFB2.

A possible explanation for Nissan's clustering lies in the geology and ecology of these islands. Both Nissan and the central Louisiades are small, low-lying coral atolls with limited habitat, relatively prone to disturbance in the form of cyclones, tidal surges, and sea-level fluctuations. Dispersal, therefore, has likely retained importance in their biology, as discussed further below. Rossel Island, on the other hand, is relatively high (838 m), large (262.5 sq km), and well forested, all characteristics that reduce the probability of island-wide disturbance and correspondingly reduce the adaptive advantage for dispersal. This hypothesis is corroborated by a more extreme example within the poorly-supported clade containing exclusively Louisiade archipelago samples (Fig. 2, node B). Here, we find several well-supported internal haplotype clades but no obvious geographic structure, suggesting even higher rates of dispersal and gene flow among clusters of tiny, low-lying islets.

Outside of the Nissan/Louisiade clade, all populations on outlying islands are phylogenetically distinct. The two samples from Rossel Island form a clade and are reciprocally monophyletic with all other populations (Fig. 2, node C). The genetic distinctiveness of this population – historically recognized as subspecies *Z. g. pallidipes* – likely reflects both its geographic isolation at the species range limit, and the unique geologic and ecological characteristics of Rossel discussed above. We find a second well-supported clade containing samples from Long Island and Nauna Island (Fig. 2, node D). While separated by the Bismarck Sea, these populations are each other's nearest neighbors, albeit relatively genetically distant (mean *p*-distance = 0.01127). Observations of open-water flight by *Z. griseotinctus* in the Bismarck Sea by Diamond and Mayr (2001) suggest occasional dispersal events between these groups, though further genetic sampling is necessary to determine the rate and frequency of gene flow.

Perhaps our most surprising result is the genetic distinctiveness of *Z. griseotinctus* populations on Long Island in the Vitiaz Strait. As mentioned, Long Island is believed to have been defaunated by a violent volcanic eruption in the 17th century (Diamond, 1974, 1998). The observed distinctiveness could be explained by three alternate hypotheses: (1) Long's eruption did not result in complete defaunation and populations retain its pre-eruption phylogenetic signal; (2) a refuge population in the vicinity of Long Island (such as islets off the west coast of New Britain) escaped extirpation and recolonized the island after the eruption; and (3) the Long Island population was subject to an extreme founder effect following recolonization after the eruption, with strong displacing selection. Given our knowledge of the extent of Long Island's destruction (Diamond et al., 1989; Ball and Johnson, 1976) and rates of molecular evolution in island birds (Lerner et al., 2011), we believe the second hypothesis is most plausible, and underscores the need for additional survey effort on small islands in Northern Melanesia.

In contrast to the relatively strong phylogenetic signal from mitochondrial DNA, nuclear locus TGFB2 is essentially invariant across populations of *Z. griseotinctus* (Fig. 3). These data provide evidence for recent divergence among populations and high gene flow, as TGFB2 is among the most rapidly-evolving nuclear introns (Lerner et al., 2011) yet shows no differentiation. Nuclear genomics techniques (such as restriction-associated DNA sequencing) may be useful in providing sufficient resolution to adequately confirm these patterns.

4.2. Evolution of the supertramp strategy

We can use our data to investigate two alternate hypotheses about the evolutionary history of supertramp species. In the first hypothesis, suggested by the conclusions of Moyle et al. (2009), isolated populations of supertramps represent nascent species, and "supertramp" status is temporary: the evolutionary lifetime of insular avian species proceeds from a period of rapid expansion to a period of diversification following a shift in dispersal ability. In this scenario, we would expect to see varied levels of divergence among *Z. griseotinctus* populations. In the second hypothesis, widespread supertramp populations remain connected by sufficient gene flow to limit divergence from genetic drift and natural selection. In this scenario, we would expect to see limited and relatively uniform divergence among populations.

We believe our mitochondrial DNA phylogeny and nuclear data best support the first hypothesis. Within the mitochondrial genome of *Z. griseotinctus*, we see both evidence of phylogeographic structure in some populations and an absence of phylogeographic structure in others, suggesting varied dispersal ability across the species' range. Additionally, an absence of differentiation in nuclear locus TGFB2 indicates recent divergence, as expected in a scenario of rapid, intraspecific shifts in dispersal ability. We believe this possible shift in dispersal ability is thus contingent on biogeographical and ecological constraints. These constraints can be seen in our phylogeny in the relatively high genetic distinctiveness of *Z. griseotinctus* outlying populations on Rossel, Long, and Nauna. As discussed above, these islands are isolated from the central Louisiade Archipelago that was best represented in our sampling. They also differ in their geology and geography: while *Z. griseotinctus* populations in the Louisiades are restricted to small coral islets between Woodlark, Sudest, Trobriand islands, and the mainland, the outlying islands are generally larger, higher, and more isolated.

Differences in size and height between islands range from relatively small (in the case of Nauna and Nissan) to several orders of magnitude (Long and Rossel). Size and height are measures that can be used as a proxy for a concept of greater island "permanence," which is expected to be inversely correlated with the likelihood an island is subject to frequent disturbance in the form of cyclones, tidal surges, and sea-level rise. The tiny, low-lying central Louisiades are particularly vulnerable to these events, while higher outlying islands are more immune (with Long Island's eruption being the most notable exception to this rule). This variation in "permanence" is central to Diamond and Mayr's (2001) proposed explanation for shifts in dispersal ability. They suggest the "supertramp" strategy is selected against on higher, more isolated islands, where disturbance events are rare and the probability of successful dispersal is lower than on low-lying islands with short interisland distances. Because *Z. griseotinctus* is found on a variety of island types, its population genetics may offer some evidence to test whether these high, isolated islands see greater divergence.

We find evidence of this selection in the greater genetic distinctiveness of outlying island populations in our phylogeny, and in our analysis of three island biogeographic models presented in Fig. 4. All three models (genetic distance from nearest neighbor population by island area, island height, and geographic distance from nearest neighbor; Fig. 4a–c) resulted in statistically significant correlations, with genetic distance from nearest neighbor population as a function of island area having the best fit ($R^2 = 0.44$), suggesting all factors may be contributing. We believe this evidence of selection against the supertramp strategy on high islands offers a plausible explanation for the rapid shifts in dispersal ability proposed in Moyle et al. (2009) to explain broad insular radiations such as White-eyes and whistlers (see Andersen et al., 2014; Jønsson et al., 2008): highly vagile taxa arrive to relatively "permanent" islands where the costs of overwater dispersal are no longer outweighed by the benefits of escaping frequent disturbance. We see additional support for this in the apparent absence of *Z. griseotinctus* on the Siassi islets southeast of Umboi (Diamond, 2015): This absence in spite of proximity to the Long Island population suggests the loss of adaptation to habitats most similar to those in the central Louisiade Archipelago.

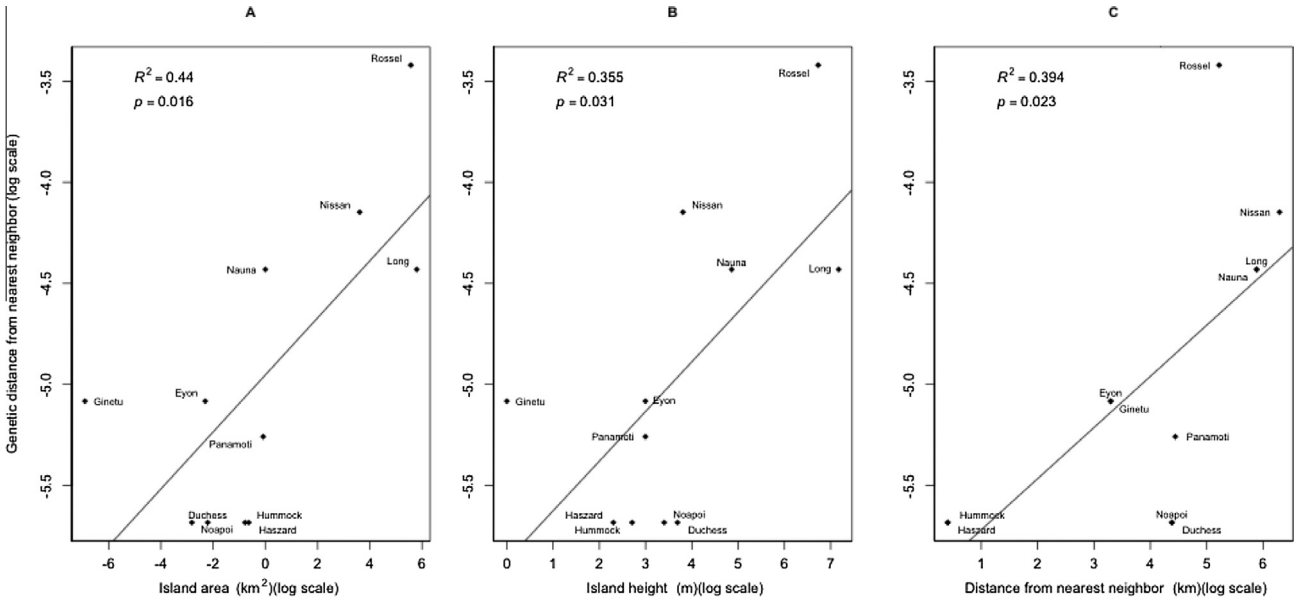

Fig. 4. Linear regressions of genetic distance between neighboring populations of *Zosterops griseotinctus* fit to three island biogeographic models. From left to right, these are genetic distance by (a) island area; (b) island height, and (c) geographic distance.

4.3. Taxonomy

Our study finds no support for the division of east/west subspecies *Z. g. griseotinctus* and *Z. g. longirostris*. Our recommendation would be to synonymize *Z. g. longirostris* with *Z. g. griseotinctus*. However, we find strong genetic evidence for the distinctiveness of outlying island populations in the phylogeny, and on basis of this and ongoing geographic isolation, populations on Rossel (*Z. g. pallidipes*), Nauna (*Z. g. ottomeyeri*), Nissan (*Z. g. eichhorni*) and Long Islands (*Z. g. sp.*) should be recognized at the subspecific level. Additional synapomorphies for the Long Island populations should be explored, and if additional support can be found, a formal name should be made available.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.08.018.

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