

1 Waves of Colonization and Gene Flow in a Great Speciator

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16 **Abstract**

17 Secondary contact between previously allopatric lineages offers a test of reproductive
18 isolating mechanisms that may have accrued in isolation. Such instances of contact can produce
19 stable hybrid zones—where reproductive isolation can further develop via reinforcement or
20 phenotypic displacement—or result in the lineages merging. Ongoing secondary contact is most
21 visible in continental systems, where steady input from parental taxa can occur readily. In
22 oceanic island systems, however, secondary contact between closely related species of birds is
23 relatively rare. When observed on sufficiently small islands, relative to population size,
24 secondary contact likely represents a recent phenomenon. Here, we examine the dynamics of a
25 group of birds whose apparent widespread hybridization influenced Ernst Mayr’s foundational
26 work on allopatric speciation: the whistlers of Fiji (Aves: *Pachycephala*). We demonstrate two
27 clear instances of secondary contact within the Fijian archipelago, one resulting in a hybrid zone
28 on a larger island, and the other resulting in a wholly admixed population on a smaller, adjacent
29 island. We leveraged low genome-wide divergence in the hybrid zone to pinpoint a single
30 genomic region associated with observed phenotypic differences. We use genomic data to
31 present a new hypothesis that emphasizes rapid plumage evolution and post-divergence gene
32 flow.

33 **Keywords:** phylogeography, island biogeography, archipelago, Pachycephalidae, speciation,
34 hybrid zone

35 Terrestrial island systems have long served as natural laboratories for studying the
36 process of allopatric speciation, where populations diverge in isolation due to limited gene flow
37 across physical barriers (i.e., water). Divergence on isolated islands tends to follow an allopatric
38 speciation model over evolutionary time (Rundell & Price 2009, Gillespie et al. 2020). Due to
39 their geographic isolation and generally small size, island populations tend to lack zones of

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secondary contact between diverging avian taxa (Kinsey 1937, Diamond 1977), like those found in well-studied continental hybrid zones (Toews et al. 2016, Brelsford et al. 2017, Wang et al. 2020). Despite this generality, sufficient reproductive isolation and ecological divergence can evolve rapidly—even between sister taxa—in more isolated islands (e.g., Darwin’s finches, Lamichhaney et al. 2015). However, islands in the Indo-Pacific that are generally more species-rich and less isolated tend to produce instances of secondary sympatry between congeneric taxa that are attributed to colonization from other archipelagos, not from within archipelagos (i.e., intra-archipelago; Diamond 1977). As such, it is rare for island taxa to occur sympatrically with their closest relative (Mayr & Diamond 2001, Andersen et al. 2015, Cowles & Uy 2019, Manthey et al. 2020). Therefore, to better understand the challenges of intra-archipelago speciation, it is important to study taxa that come into secondary contact with previously isolated congeners.

Natural invasions between allopatric island lineages have rarely been witnessed. As such, there are few avian examples where secondary contact produces traditional hybrid zones on islands (Graves 2015, Sardell & Uy 2016). Instead, hybridization in island birds tends to be expressed as hybrid populations, an observation first made from careful study of museum specimens collected across Melanesia (Mayr 1932a, 1932b, 1938, 1942; Mayr & Diamond 2001). Recent genetic studies have provided further support for this phenomenon in islands and in other isolated areas of secondary contact (Nietlisbach et al. 2013, Lavretsky et al. 2015, Barrera-Guzmán et al. 2017, Andersen et al. 2021, Colella et al. 2021, McCullough et al. 2021). This is because islands lack the spatial scale to host a full hybrid zone and lack consistent parental input required for early generation hybrids (Kinsey 1937, Mayr 1942, Diamond 1977, Kissel and Barraclough 2010). Several aspects of island geography further facilitate the formation of hybrid populations. For example, the higher number of migrants originating from larger islands and continents can mean that smaller islands adjacent to larger ones are especially prone to the formation of hybrid populations (see MacArthur & Wilson 1963, 1967; Gyllenhaal et al. 2020). Additionally, glacial cycles can cause cyclical connectivity of islands and thereby increase rates of gene flow between island populations (Brown et al. 2013, Tan et al. 2022). This phenomenon of glacial eustasy could promote the formation of island hybrid zones, but as such cycles have occurred for millennia, present-day insular hybrid zones likely represent a deviation from longer-term isolation.

A conspicuous element of island bird faunas, especially in the Indo-Pacific, is the abundance of widespread ‘polytypic’ species. These geographic radiations occur on many islands—often across multiple archipelagos—and although apparently closely related, each island population may differ markedly in plumage pattern or coloration. *Pachycephala* whistlers are amongst the most widespread and strikingly diverse geographic radiation across the Indo-Pacific (Galbraith 1956) and Ernst Mayr leveraged this ‘great speciator’ lineage to inform our early understanding of the process of allopatric speciation (Mayr 1932a, 1942, 1963; Mayr & Provine 1980). Most insular populations of *Pachycephala* are allopatric, but there are several instances of secondary contact between divergent lineages, which has led to outcomes that range from secondary sympatry to apparent hybrid populations (Mayr 1932a, Mayr 1932b, Mayr & Diamond 2001).

The Fiji Whistler (*Pachycephala vitiensis*) is a monophyletic lineage of 10 named taxa (Andersen et al. 2014; Jönsson et al. 2014), which is part of the broader geographic radiation

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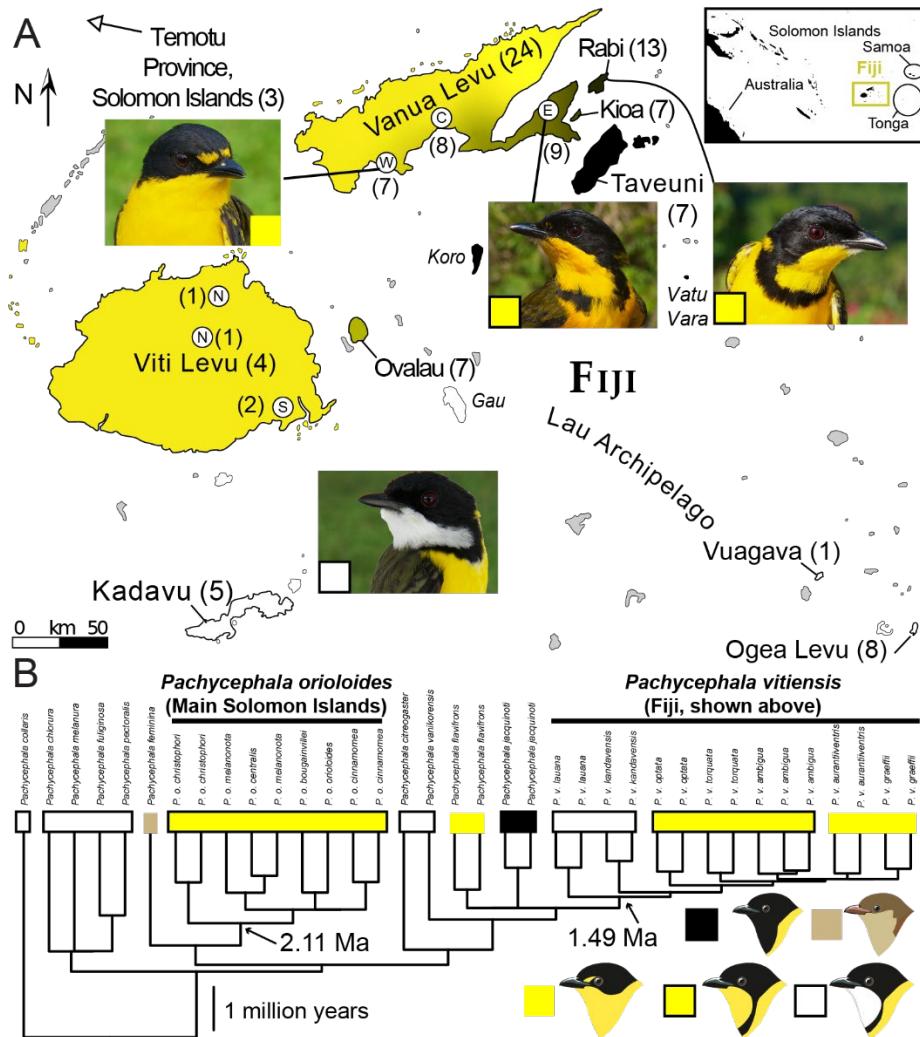


Figure 1: A) Map of islands sampled and a representation of plumage coloration. Islands with unknown plumage coloration are gray. Sampled islands are labeled by name and have a sample number. Unsampled islands with distinct taxa are labeled in italics. Islands without whistlers are gray, and island color corresponds with phenotype. White represents males with a white throat and black breast band, black represents a yellow throat and black breast band, yellow represents a yellow throat and no breast band, and intermediates between black and yellow represent rough frequencies of black breast band. Photos of archetypical individuals are shown, with a box corresponding with the color scheme in B. Sampling points (white circles) on large islands are shown, and when relevant the abbreviated cardinal direction is used to discuss samples from given points. Inset depicts the location of Fiji relative to other areas of interest. B) Phylogeny inferred from ultraconserved elements for more whistler species used for estimating divergence dates. Tips have boxes corresponding to male plumage patterns, with cartoon examples of phenotypes in the bottom right corner. Mean crown divergence times inferred by BEAST are at relevant nodes. Artwork by Jenna McCullough, some with modifications, based on artwork from Birds of the World (Billerman et al. 2020).

84 known as the Golden Whistler species complex (Galbreath 1956, 1967). Most taxa are single-
85 island endemic populations that are distinct in appearance; yet, their distinctiveness consists of

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86 variations on a theme. All golden whistlers have dark backs and yellow underparts, but
87 populations differ in combinations of throat color (white or yellow), the presence or absence of a
88 black band across the breast, and other minor plumage details. Thus, three main types of
89 populations exist: white-throated with a black breast band, yellow-throated with a black band,
90 and yellow-throated with no band (Fig. 1). Typically, one such phenotype occurs across an
91 archipelago (e.g., *P. citreogaster* in the Bismarck Archipelago or *P. chlorura* in Vanuatu);
92 however, all three plumage types are represented within Fiji, and Mayr hypothesized that the Fiji
93 Whistler represented ongoing contact between an old, yellow-throated lineage and a newly
94 expanding, white-throated lineage. This hypothesis featured prominently in some of his
95 influential works (e.g., Mayr 1942, Mayr 1963), especially how the failed secondary contact of
96 this and other whistlers highlight the importance of reproductive isolation in permitting
97 sympatry. Moreover, this lent support to his conclusion that allopatric lineages such as these do
98 not represent biological species (Mayr 1932a). It was also used to support the notion that islands
99 facilitate the formation of hybrid populations (Mayr 1942). Here, we use genomic data to test
100 Mayr's hypothesis of colonization history and assess the nature of admixture upon secondary
101 contact between two phenotypically divergent lineages on Vanua Levu and its outlying islands in
102 Fiji (Fig. 1A). We tackle these questions using three genomic datasets: restriction site-associated
103 DNA sequencing (RAD-seq) of the focal Fijian taxa, target capture data of a broader range of
104 taxa to determine the age of the Fijian radiation, and a new draft reference genome.

105 Methods

106 Sampling and DNA Extraction

107 We collected genetic data from all major phenotypes in the Fijian archipelago, with an
108 emphasis on the contact zone across Vanua Levu (Fig. 1). We sampled 79 individuals of the
109 genus *Pachycephala* for our primary RAD dataset (excluding samples with too few reads or too
110 high relatedness), of which 76 comprised our core *P. vitiensis* sampling, and a non-mutually
111 exclusive set of 63 samples comprised our yellow-throated ingroup sampling (Fig. 1). All
112 individuals were represented by tissues with voucherized specimens (Table S1). For yellow-
113 throated Fijian taxa (formerly *Pachycephala graeffii*), we sampled seven from Ovalau (*P. v.
114 optata*), four from Viti Levu (*P. v. graeffii*), 24 from Vanua Levu (*P. v. aurantiiventris* and
115 *ambigua*), 13 from Rabi (*P. v. ambigua*), seven from Kioa (*P. v. ambigua*), and seven from
116 Taveuni (*P. v. torquata*). For white-throated taxa, we sampled five from Kadavu (*P. v.
117 kandavensis*), eight from Ogea Levu (*P. v. lauana*), one from Vuagava (*P. v. lauana*), and three
118 from Nendo (Solomon Islands; *P. vanikorensis ornata*). We lacked three single-island,
119 subspecific populations of *P. vitiensis*: the yellow-throated *P. v. bella* and *P. v. koroana* from
120 Vatu Vara and Koro, respectively, and the white-throated *P. v. vitiensis* from Gau. In addition to
121 our primary RAD dataset, we used one sample of *P. v. kandavensis* (KU 117379) to generate a
122 nuclear reference genome assembly using linked-read sequencing on the 10X chromium
123 platform. Finally, we compiled a dataset of ultraconserved elements (UCEs; Faircloth et al.
124 2012) from 36 individuals across the *P. pectoralis* superspecies (Table S1). This UCE dataset
125 represents an expansion of Clade A from Brady et al. (2022) and is composed of 24 newly
126 sequenced samples and 12 samples sequenced for Brady et al. (2022). Four of these samples
127 were derived from museum specimen toepads (two each of *P. jacquinoti* and *P. flavifrons*), while
128 the rest were from specimen-voucherized tissues (Table S1).

129 Reduced-representation Bioinformatics

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130 We used Stacks v2.4.1 (Rochette et al. 2019) to call single nucleotide polymorphisms
131 (SNPs) for our focal RADseq dataset and used the outputs as input for downstream analyses. We
132 used the *process_radtags* module in single-end mode for demultiplexing reads by individual,
133 which we then aligned to our reference genome using the *mem* algorithm of BWA v0.7.17 (Li &
134 Durbin 2009). We used the Stacks module *gstacks* to assemble RAD loci based on these
135 alignments and call SNPs. We generated multiple datasets using *gstacks* for different analyses
136 and used the *gstacks* output to generate input files using *populations*. Unless otherwise stated, we
137 performed these analyses with a 75% complete SNP matrix (i.e., SNPs present in 75% of
138 individuals were included). We processed UCEs using the phyluce v1.7.0 pipeline (Faircloth
139 2015), following exact details as outlined in Brady et al. 2022.

140 *Population structure*

141 We used several methods to explore population structure at all levels using RAD data,
142 but with a focus on yellow-throated *P. vitiensis*. First, we calculated Weir and Cockerham's
143 F_{ST} between each sampling region with more than one individual (island or sub-region of
144 island if relevant) using a custom wrapper around vcftools v0.1.15 (Danecek et al. 2011).
145 Although Weir and Cockerham's estimator can be biased slightly for low sample sizes at higher
146 levels of divergence, this is not relevant for focal comparisons (Willing et al. 2012). This script
147 was first generated for Mapel et al. (2021), and an updated version can be found on GitHub:
148 <https://github.com/ethangyllenhaal/FijiPachyRad>. This script was run separately for loci assigned
149 to autosomes and the Z chromosome. Second, we ran principal component analyses (PCAs)
150 using the *glPca* module of adegenet v2.1.1 (Jombart 2008) in R v3.6.1 (R Core Team, 2019),
151 which uses imputation to address missing data. Input was generated using the R package *vcfR*
152 v1.8.0 (Knaus and Grünwald, 2017). Finally, we evaluated admixture proportions of yellow-
153 throated *P. vitiensis* with sNMF (Frichot et al. 2014) in the R package *LEA* v2.2.0 (Frichot &
154 François, 2015). Input for sNMF excluded uninformative singletons (i.e., minor allele count <2;
155 Linck & Battey 2019). The alpha (normalization) parameter was set to 10 after exploratory runs
156 of what minimized the cross-entropy criterion, but the results were similar across different
157 values. The result with the minimum cross-entropy criterion after 50 iterations per k value was
158 chosen for plotting.

159 *Phylogenetic trees and networks*

160 We generated a phylogeny of our RAD data using a concatenated maximum likelihood
161 approach (treating heterozygotes as equal likelihoods of either nucleotide) with IQ-TREE v2.8.3
162 (Minh et al. 2020), using ModelFinder (Kalyaanamoorthy et al. 2017) for model selection with
163 1000 ultrafast bootstraps (Hoang et al. 2018) for assessing support. We used individual-level,
164 *phylip*-formatted data generated from a 90% complete matrix. Two samples presented as
165 problematic (unexpected location based on other analyses, reducing support value of intervening
166 samples) and were removed from subsequent sequence-based analyses. We also estimated a
167 species tree using SVDQuartets in PAUP* v4.0a169 with 100 bootstraps and evaluating 100,000
168 quartets (Chifman & Kubatko 2014), grouping samples by sampling region (Fig. 1). To estimate
169 the age of our focal radiation, divergence time dating was performed in BEAST v2.6.7
170 (Bouckaert et al., 2019; Appendix 1). To account for major introgression events, we generated a
171 population tree with inferred admixture events using TreeMix v1.13 (Pickrell & Pritchard 2012).
172 This analysis was conducted with a 90% complete matrix of SNPs to limit the effects of missing
173 data. We iteratively added migration edges until the last meaningful edge was added that

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174 increased the likelihood by more than two (equivalent to a change of the Akaike Information
175 Criterion of four, a common threshold for model selection).

176 *Tests for gene flow*

177 We performed statistical tests for gene flow with ABBA/BABA tests as implemented in
178 Dsuite v0.4r38 (Malinsky et al. 2021) with *P. vanikorensis* as an outgroup, with a 90% complete
179 VCF as input. We calculated the D statistic (Durand et al. 2011) and its significance in addition
180 to the f_4 estimate of admixture proportion (Patterson et al. 2012). We focused on two *a priori*
181 hypotheses: 1) gene flow between populations on Vanua Levu and those on nearby islands and
182 2) gene flow between white- and yellow-throated *P. vitiensis*. We also used the f_{branch} function to
183 make inferences about timing of gene flow (Malinski et al. 2018). We took three approaches to
184 account for multiple comparisons, described in Appendix 1 (Supplemental Methods; Correcting
185 for multiple tests for gene flow).

186 *Genome scans*

187 To understand the genomic architecture of phenotypic divergence, we estimated Weir and
188 Cockerham's (1984) weighted F_{ST} in 10 kbp windows across the genome using vcftools v0.1.15
189 (Danecek et al. 2011) and plotted the results using the R package qqman v0.1.8 (Turner 2018). In
190 most cases (but not all), this windowed value is the mean from one RAD locus; windows without
191 SNPs are ignored. We took two approaches to assign comparisons: geographic locality and
192 phenotype. For the former, this represents a standard inter-taxon F_{ST} outlier comparison, and the
193 latter is closer to a GWAS. The phenotypic indices were estimated by MJA, and included indices
194 for the extent of yellow in the lores and black on the breast of yellow-throated *P. vitiensis*. In
195 short, the extent of the band was binned into four categories (absent, hooked, dirty, and
196 complete; 0–3 respectively) and lores into three (black, minimally yellow, and completely
197 yellow; 0–2 respectively). We assessed putative functional genes based on our annotations
198 (Supplemental Methods) for windows with F_{ST} greater than 0.5 in the eastern vs western Vanua
199 Levu, comparison, including a region bounded by values >0.5 on the Z chromosome.

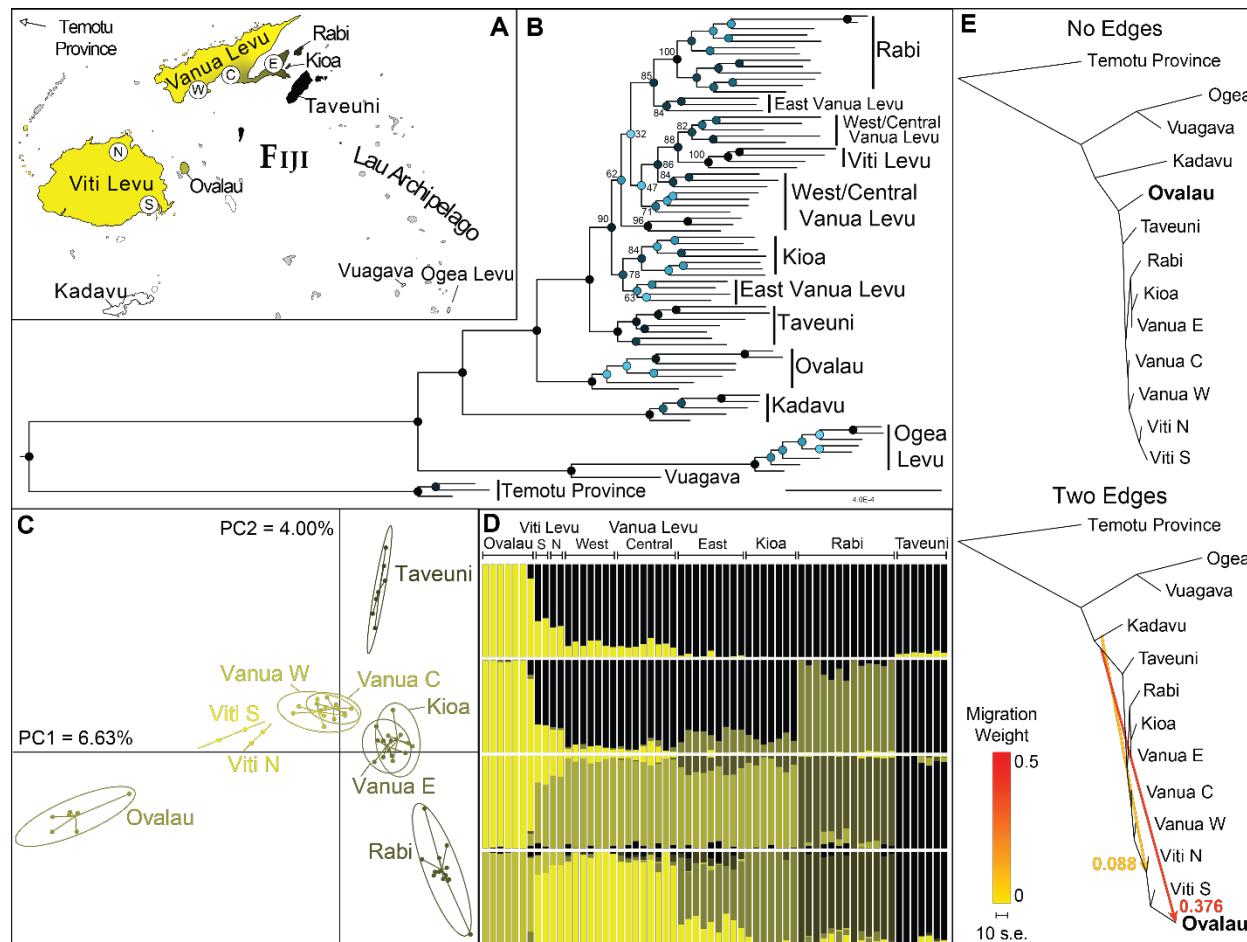
200 **Results**

201 *Population structure*

202 Each of the three white-throated *P. vitiensis* populations formed distinct genomic
203 clusters, all of which were notably distinct from the yellow-throated populations (Fig. S1).
204 Within the yellow-throated taxa, there was notable population structure corresponding to
205 geography and the extent of dark plumage in the breast and lores of a given population (Fig. 2C–
206 D, S3). In principal component space, more positive values of PC1 largely corresponded to
207 darker populations. The one exception was the population on Ovalau, which has intermediate
208 plumage but was separated in PC space from all other yellow-throated populations (Fig. 2C–D).
209 Populations from Kioa and eastern Vanua Levu clustered together, as did populations from
210 central and western Vanua Levu. Estimates of admixture fractions tended to be primarily from
211 only one ancestral population, but notable levels of mixed ancestry were consistently inferred for
212 eastern Vanua Levu (Fig. 2D, S3). Estimates of genome-wide F_{ST} across Vanua Levu were
213 associated with plumage coloration. For example, with the western Vanua Levu populations as a
214 reference, F_{ST} increased moving east across Vanua Levu, with a further increase on the darker
215 island populations on Kioa, Rabi, and Taveuni (Table S3). F_{ST} was generally higher on Z-

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216 chromosomes than on autosomes, except for the comparison between Kioa and eastern Vanua
 217 Levu.



218
 Figure 2: A) Map of focal sampling localities in Fiji. North, South, East, West, and Central are abbreviated to N, S, E, W, and C, respectively. B) IQ-TREE Phylogeny of RAD-seq dataset, with groups of tips labeled corresponding to the inset map. Node shapes correspond to support value, with lighter values indicating lower support. Support values for potentially geographically informative nodes in the main yellow-throated clade are shown, but we note relationships among the main yellow-throated clade are largely unresolved. C) Genomic PCA of all yellow-throated taxa, with order of colors on a yellow-black gradient corresponding to the relative mean band extent in the population (more extensive bands in blacker clusters). D) Ancestry plots from sNMF analyses of yellow-throated taxa for K values of 2-5, colored as C. E) TreeMix Phylogenetic networks with zero and two migration edges, with labels corresponding to the weight of the edge (see Figure S3 for one edge network).

219 *Phylogenetic trees and networks*

220 Our phylogeny inferred in IQ-TREE recovered the white-throated Lau populations as
 221 sister to other *P. vitiensis*, with the population on Kadavu sister to all yellow-throated taxa (Fig.
 222 2B, Figure S2). Within the yellow-throated populations, Ovalau individuals were sister to all
 223 others, then Taveuni was moderately (bs= 90) supported as sister to the remaining yellow-

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224 throated clade. Support values for the rest of the tree were low, and many populations that
225 clustered in PCA space were not monophyletic. Populations that were fully supported as
226 monophyletic were from the relatively isolated islands of Rabi and Viti Levu. The phylogenetic
227 network from TreeMix recovered a similar topology when no migration edges were added (Fig.
228 2E, S3). However, when migration was modeled, a significant migration edge was inferred
229 between Ovalau and an ancestral taxon (admixture fraction=0.376; $p < 0.0001$). In turn, Ovalau's
230 placement in the tree changed to be sister to the southern Viti Levu population and nested within
231 the clade of populations with weaker breast bands (Fig. 2E). A second, less significant ($p =$
232 0.0067), edge was inferred between an ancestral Kadavu population and the ancestor of the Viti
233 Levu clade when two edges were considered (Fig. 2E). Our species tree analysis in SVDQuartets
234 produced some similar results but was very sensitive to the inclusion of the Ovalau population
235 (Fig. S4). Although several nodes were decently supported without it (often more so than in IQ-
236 TREE), the putatively admixed population resulted in high topological uncertainty when it was
237 included. Our UCE time tree (Fig. 1B) suggested that the crown age of Fijian whistlers is
238 approximately 0.75–2.58 Ma (mean 1.49 Ma) and that mean ages of island-specific taxa in Fiji
239 range from 0.66–0.96 Ma. Within the Solomons Whistler (*P. orioloides*), there was general
240 support for clades corresponding to geologically cohesive archipelagos within the broader
241 Solomons Archipelago—the New Georgia Group, the Bukida Group, and Makira Island
242 (excluding *P. feminina*)—which had an inferred crown age of 0.89–3.28 Ma (mean 2.11 Ma).
243 The UCE topology was similar for the Fijian clade despite lower sampling (Fig. S5).

244 *Tests for gene flow*

245 Tests for gene flow in the group provided evidence for widespread gene flow throughout
246 the archipelago, although comparisons within closely related groups were not possible due to the
247 constraints of ABBA/BABA tests. The significant tests ($p < 0.05$) between adjacent populations
248 are outlined in Figure 3A. All tests are in Table S3, and f_{branch} in Figure S6. In short, high levels
249 of gene flow (f_4 admixture fraction > 0.2) were inferred between Kioa and western and central
250 Vanua Levu, Rabi and points west, Taveuni and both Rabi and Kioa, and Ovalau and Viti Levu.
251 The latter was highly significant, likely due to the admixed nature of Ovalau, and resulted in
252 unusual topologies being tested. All of these were significant after accounting for multiple
253 comparisons except for gene flow between Rabi and points west (harmonic $p=0.068$) and
254 between eastern Vanua Levu and the rest of Vanua Levu (harmonic $p=0.053$). However, the
255 latter is supported by phenotypic evidence. Low levels of gene flow were inferred between Viti
256 Levu and Kadavu and between Ogea Levu and several other populations (Viti Levu, central
257 Vanua Levu, Rabi, and Kioa). These results are not robust to multiple comparisons, but gene
258 flow between Viti Levu and Kadavu (or a related population) is supported by TreeMix (Fig. 2E).

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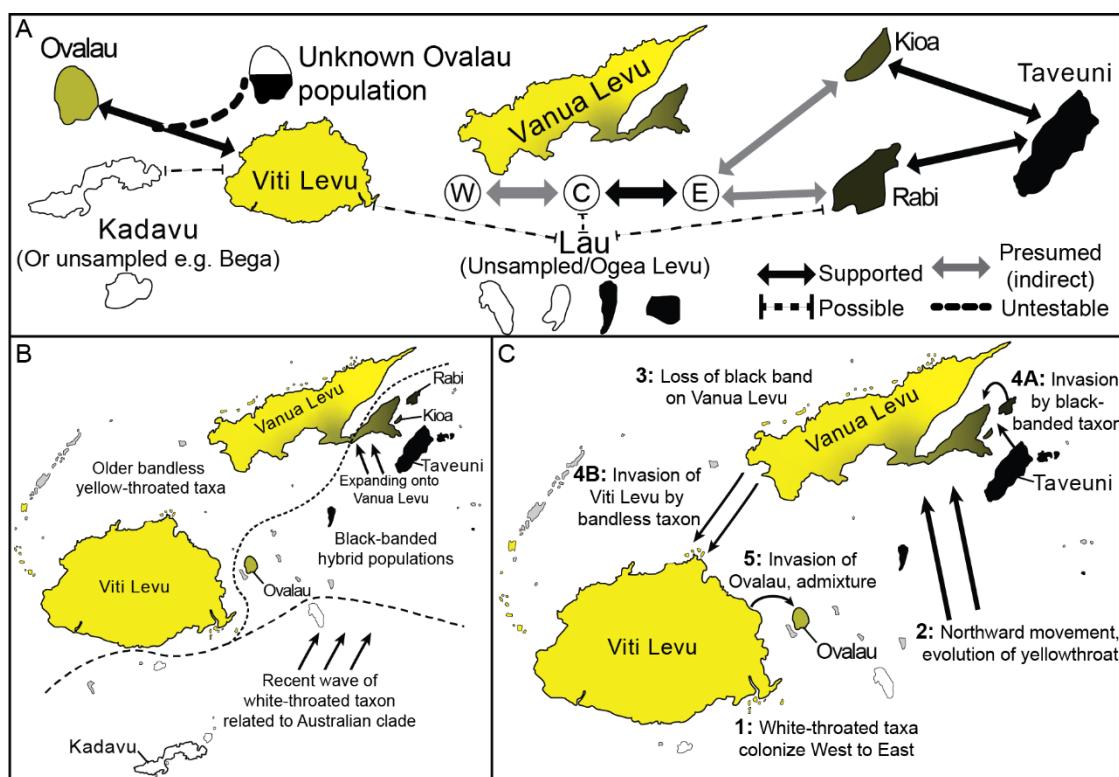


Figure 3. A) Sampled populations (represented by their respective islands' shape and phenotype) with arrows corresponding to admixture fraction as estimated by the f_4 ratio. The “possible” line denotes edges with significant gene flow for some tests but did not stand up to multiple comparisons. “Presumed” edges represent cases where gene flow is readily testable, but would have occurred based on gene flow inferred between non-adjacent populations (e.g., between Rabi and Western Vanua Levu). Note that the islands are not drawn to scale and are arranged to illustrate the linear pattern of inter-island gene flow. B-C) Diagrams of Mayr's (B) and our (C) hypothesized colonization history of whistlers in Fiji.

260

Genome scans

261 Our exploration of the genomic architecture of divergence found one consistent (multiple
 262 consecutive windows with high F_{ST} in the eastern vs western Vanua Levu comparison) peak
 263 differentiating darker and yellower populations, based on both geography and hybrid index. This
 264 peak resided on the Z chromosome and was clearest between the populations with the most
 265 similar genome-wide F_{ST} but differing band extracts (eastern vs western Vanua Levu; Figure 4).
 266 Several other peaks between Taveuni and other populations were also found, including a broader
 267 peak on the Z chromosome (Fig. S7). Our assessment of potential causal loci found two genes
 268 that have been correlated in plumage or pigmentation in past studies. One gene, TRPM7, is in the
 269 same family as TRPM1, which has been correlated with feather pigmentation in domesticated
 270 chickens. More notably, PCSK5 has been directly correlated with variation in melanin levels in
 271 wild birds, and is associated with melanin production pathways (San-Jose et al. 2017). However,
 272 several genes associated with other phenotypes such as fat deposition (NR2F2; Zhu et al. 2021)
 273 and stress responses (MCTP1; Taff et al. 2019) were also in this large region.

274

Discussion

275 The genus *Pachycephala*, particularly those in Fiji, had a major impact on the
276 development of Ernst Mayr's view of allopatric speciation during the Modern Synthesis (Mayr
277 1932a, Mayr 1932b, 1942). He interpreted phenotypic evidence to suggest multiple instances of
278 hybrid populations after phenotypic divergence in allopatry, representing the failure of such
279 lineages to attain reproductive isolation despite having plumage traits as different as some
280 continental sympatric species in the same genus (e.g., *P. soror* and *P. schlegelii*, *P. pectoralis*
281 and *P. melanura*). Our study supports the general idea that these birds failed to speciate
282 completely when in allopatry, but we lacked support for Mayr's exact hypothesis for how this
283 occurred geographically (Fig. 3B). We instead present a hypothesis that emphasizes rapid
284 plumage evolution occurring in geographic allopatry as the species colonized Fiji, producing
285 much greater diversity in male plumage than seen in the older radiation in the Solomon Islands
286 (Fig. 3C). However, a second wave of movement—likely facilitated by glacial cycles—resulted
287 in secondary contact, which caused the formation of a hybrid population on Ovalau (Fig. 2, 3)
288 and a series of intermediate populations across Vanua Levu (Fig. 2). We also find evidence that
289 the phenotypic divergence in the Vanua Levu populations is largely tied to one region of the Z
290 chromosome, rather than at several points across the genome.

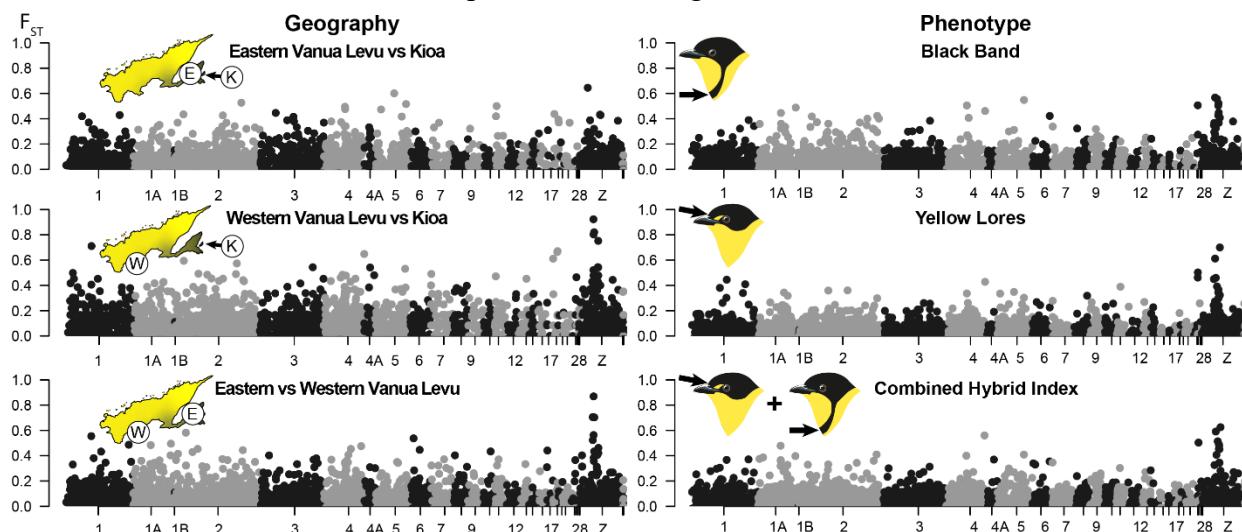


Figure 4. Manhattan plot of F_{ST} across the whole genome (based on synteny mapping with Zebra Finch). The left column compares by population (with East Vanua Levu and Kioa sharing similar phenotypes), while the right compares by phenotype. For the right column, the values compares are band values of 0–1 versus 2–3, lobe values of 0 versus 1–2, and combined index values of 0–1 versus 3–5 (see Methods; Genome Scans).

291

Secondary contact of allospecies

292 Our dataset included two instances of secondary contact, the contact zone on Vanua Levu
293 and a genetically confirmed hybrid population on Ovalau, both recognized by Mayr as potential
294 instances of contact between allospecies (Mayr 1932a). The Vanua Levu contact zone is between
295 the two yellow-throated phenotypes, where a black-banded population invaded un-banded
296 populations from outlying islands to the East (i.e., Kioa and Rabi), with high direct or indirect
297 gene flow inferred between all sampled populations in the region (Fig. 4a). We did not find a
298 clean clinal pattern, but rather a divide in phenotype and genotype between the two western

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299 Vanua Levu populations and eastern Vanua Levu plus Kioa. This suggests that further sampling
300 of the southern coast of Vanua Levu is required to fully understand the hybridization dynamics
301 in this group, but our detection of high levels of gene flow and low genetic divergence across the
302 island suggests high admixture (Fig. 2, 3a; Table S3). The narrow stretch of land between the
303 eastern and central sampled populations may have caused this relatively steep transition, and
304 indeed may facilitate the long term persistence of a hybrid zone, as is seen in some continental
305 contact zones (e.g., Cicero et al. 2023).

306 For Ovalau, a population noted by Mayr to harbor extensive phenotypic variability (Mayr
307 1932a), we find evidence for a lineage merger between an un-banded, yellow-throated
308 population from Viti Levu and an unsampled or ghost taxon that likely originally occupied the
309 island prior to this merger (Fig. 3, 4). Traditional concatenated and species tree analyses
310 struggled with this taxon, either placing it as sister to other yellow-throated taxa with full support
311 (Fig. 2) or resulting in inflated topological uncertainty (Fig. S4), respectively. TreeMix, which
312 allows the inference of admixture events, resulted in a major topological rearrangement.
313 Andersen et al. (2021; described further in McCullough et al. 2021) found a similar result in a
314 strikingly similar geographic context, an isolated island fully or nearly connected during glacial
315 periods, which along with other findings could suggest this is a frequency pattern in island taxa
316 (Nietlisbach et al. 2013, Lavretsky et al. 2015, Colella et al. 2021). Much like the example in
317 Andersen et al. (2021), the phenotype of the original Ovalau lineage is not fully known. It could
318 either be a yellow-throated, black-banded population like those found on the unsampled island of
319 Koro, or a white-throated, black-banded population like those found on Beqa and Gau islands. A
320 better-documented hybrid population between yellow-throated and white-throated taxa has many
321 individuals with intermediate throat color unlike the pure yellow throat of Ovalau individuals
322 (Mayr 1932b). Further sampling should reveal whether this represents a population akin to
323 Mayr's hypothesis for the genetic origin of all black-banded birds, where hybridization between
324 un-banded and white-throated populations produces the yellow-throated, black-banded
325 phenotype that we sampled from islands east of Vanua Levu (Fig. 1A; Mayr 1932a, 1942).
326 However, we found no evidence that the majority of yellow-throated, black-banded taxa
327 represent hybrid populations (Fig. 3).

328 Genomic architecture of allospecies divergence

329 We detected only a single multi-locus divergent genomic region in the instance of
330 secondary contact on Vanua Levu (Fig. 4). This Z-linked region stood out the most strongly
331 when only geography (i.e., western Vanua Levu compared to Kioa) was considered, but was
332 evident when phenotype was considered as well. Although one gene in this region (PCSK5) has
333 mechanistic ties to plumage divergence (San-Jose et al. 2017), it is part of a larger region full of
334 genes involved in other pathways. Although it is tempting to ascribe causality to this gene, we
335 believe further validation is required to rule out hypotheses such as genetic hitchhiking. Indeed,
336 even if it is causal, the phenotype itself may not have evolved under strong selection, but due to
337 alternatives such as hitchhiking off selection on a linked gene or drift (Gould & Lewinton 1979,
338 Barton 2000). Regardless, inter-population divergence being linked to a single chromosomal
339 region is consistent with genome-wide admixture proportions matching geography rather than
340 within-population phenotype (Fig. 2C). Additionally, when the darkest birds on Taveuni were
341 compared to other populations, including the relatively dark individuals on Rabi, a wider peak in
342 the same chromosomal region was observed (Fig. S7). Future work can assess if reduced
343 recombination across lineages drove divergence in this region, adaptive or not. The low number

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of genomic regions involved in divergence is also consistent with the apparent lack of resistance to gene flow, as even if selective forces shape this divergence, polygenic adaptation better promotes cohesion during secondary contact (Barton 1983, Barton & Hewitt 1985, Flaxman et al. 2014). If this region is broadly responsible for the similar plumage diversity in other whistlers, it may suggest a model where great speciators evolve high plumage divergence despite minimal intrinsic reproductive isolation or ecological specialization.

350 History of colonization and plumage divergence

351 Our results provide support for two themes of Mayr’s hypothesis: dynamic waves of
 352 colonization and the lack of reproductive isolation between allopatric lineages (Mayr 1932a,
 353 1942). However, the underlying dynamics of colonization that we inferred differed starkly from
 354 Mayr’s interpretation. Mayr hypothesized that recent expansion of white-throated taxa into the
 355 Fijian archipelago from Vanuatu (*P. chlorura*), resulting in the introgression of a dark-banded
 356 phenotype into an unbanded, yellow-throated population and the formation of hybrid populations
 357 of banded, yellow-throated birds (Mayr 1932a). We found no evidence of recent contact between
 358 “old” and “new” phenotypes. This is evidenced by the monophyly of yellow-throated birds and
 359 the nested nature of less banded birds in our phylogeny (Fig. 3). Indeed, the proposed white-
 360 throated lineage does not represent a monophyletic group, but rather a laddered series of white-
 361 throated taxa leading to the yellow-throated clade. We also found no evidence that banded,
 362 yellow-throated populations, specifically those on Taveuni, shared an excess of alleles with
 363 white-throated lineages (Fig. 3C, 4A, S4).

We propose an alternative model for the colonization of Fiji whistler. First, a white-throated lineage underwent an initial wave of colonization of the Fijian archipelago, followed by the evolution of both yellow-throated phenotypes and a second wave of dispersal among entirely (or mostly) yellow-throated populations, which led to the two admixture scenarios outlined above (Fig. 4C). The phylogenetically nested nature of yellow-throated Fijian populations within a clade of white-throated Kadavu and Lau populations (Fig. 1A, 3B) suggests a wave of colonization by a dispersive, initially white-throated lineage throughout isolated islands of Fiji. Second, the loss of a white throat from northern and western Fijian populations suggests a single evolution of a yellow-throated phenotype during the northward colonization of the archipelago. Third, the nested nature of unbanded populations suggests the loss of the black band while outlying islands (e.g., Koro, Taveuni, and Rabi) retained their banded phenotype. Fourth, we propose a recent wave of admixture driven by glacial cycles resulting in increased propagule pressure from Rabi and Kioa to the Natewa Peninsula of Vanua Levu—which is relatively isolated from the rest of the island due to the long, narrow base of the peninsula—resulting in a zone of phenotypic intermediates. The glacial cycles then also resulted in the colonization of Viti Levu by this clean-breasted form, possibly filling an empty niche or displacing and genetically swamping a white-throated ghost population related to birds on Kadavu (resulting in a signal of gene flow, Figure 3C and 4A). This last point is supported by the close relationship between Viti Levu and western Vanua Levu populations, despite the long over-water distance during interglacial periods, and continued separation during glacial periods (Fig. 2, Table S2). Finally, during this wave or during glacial maxima, the island of Ovalau, was partially genetically swamped by the new clean-breasted lineage that had colonized Viti Levu. We believe that the relatively distinct nature of Ovalau, an island that was fully connected to Viti Levu during glacial maxima, is consistent with this admixture occurring during a recent glacial cycle, as the propagule pressure from extended contact would likely fully erode the genetic and phenotypic

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389 signatures of the population's admixed origin. Indeed, once gene flow is accounted for, Ovalau is
390 nested within the Viti Levu populations (Fig. 3C). Estimating the timing of these events in a
391 phylogenetic context is complicated by intraspecific divergence (Fig. 1B) and confounding gene
392 flow (Fig. 4). However, our proposed historical model is supported by multiple lines of genetic
393 evidence and is parsimonious with respect to the number of colonization waves and amount of
394 gene flow between extant taxa.

395 This colonization pattern is unlike another taxon with a similar distribution, the wattled
396 honeyeaters (*Foulehaio*). In this case, older lineages occupy the larger islands of Fiji, while more
397 isolated islands in the Lau, Tongan, and Samoan archipelagos are recently diverged with
398 evidence of long-distance gene flow between them (Mapel et al. 2021). Instead, *Pachycephala*
399 illustrates the opposite pattern, with populations in Lau, Tonga, and Samoa sister to the
400 populations on the larger islands (Fig. 1b, 2). However, the genetic and phenotypic affiliation
401 between the Natewa Peninsula and Taveuni is consistent with the endemism of *Lamprolia*
402 silktails (Aves: Rhipiduridae)—a deeply divergent relictual lineage—to those two regions
403 (Irestedt et al. 2008, Andersen et al. 2017). These observations highlight that although we can
404 make some predictions about how recently diverged, allopatric populations will interact upon
405 secondary contact, colonization history can be highly idiosyncratic between co-distributed
406 lineages.

407 Conclusion

408 Our work revisited a system that had a disproportionately large influence on Mayr's
409 contributions to the Modern Synthesis and the study of speciation (Mayr 1942). Fiji whistlers
410 feature many important elements of speciation theory. Most relevant to Mayr's implementation
411 on the Biological Species Concept, we found a lack of evidence for reproductive isolation of
412 allopatric taxa despite having phenotypic divergence comparable to sympatric species (Mayr
413 1942). Relating to the study of the genomic architecture of speciation, we found one instance of
414 secondary contact that resulted in high introgression across Vanua Levu (Fig. 4A) demonstrated
415 the weakness of single genomic regions in producing strong reproductive isolation (Barton 1983,
416 Flaxman et al. 2014). Finally, as investigators have increasingly demonstrated in recent decades,
417 geographic variation in plumage coloration can evolve rapidly, with two major transitions in
418 plumage phenotype occurring in a short span of time (approximately 500,000 years). This is
419 especially impressive compared to the *P. orioloides* radiation in the Solomon Islands, which has
420 mostly undergone changes in the extent of black plumage over a longer period of time than it
421 took for *P. vitiensis* to evolve its wide range of phenotypes (Fig. 1B). Our study posits a new
422 biogeographic hypothesis for the Fiji whistler and establishes a framework to inform future work
423 on the genomic basis of color evolution in this group. Finally, our study highlights the value of
424 historic and modern collections to reexamine long-standing hypotheses about how species form
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446 **Data Accessibility Statement**

447 Population genetic input files, phylogenetic trees and input files, and reference genomes will be
448 uploaded to Dryad: <https://doi.org/10.5061/dryad.k98sf7mft>. Scripts are archived on Zenodo,
449 linked to the Dryad repository, as well as on a personal GitHub:
450 <https://github.com/ethangyllenhaal/FijiPachyRad>. Raw Illumina sequencing reads for RAD-seq
451 and new UCE data are available from the NCBI SRA (BioProject PRJNA1088558).

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