Waves of Colonization and Gene Flow in a Great Speciator

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Abstract

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

Keywords: phylogeography, island biogeography, archipelago, Pachycephalidae, speciation, hybrid zone

Terrestrial island systems have long served as natural laboratories for studying the process of allopatric speciation, where populations diverge in isolation due to limited gene flow across physical barriers. Over evolutionary timescales, divergence on isolated islands often follows an allopatric speciation model (Rundell & Price 2009, Gillespie et al. 2020). In more isolated, species-poor islands such as the Galapagos, reproductive isolation and ecological divergence can evolve rapidly within an archipelago (e.g., Darwin's finches, Lamichhaney et al. 2015). The more species-rich islands of the Indo-Pacific tend to only have congeneric sympatric avian taxa that are the result of colonization from other archipelagos, rather than diversification within an archipelago (i.e., intra-archipelago speciation; Diamond 1977). Consequently, it is rare for Indo-Pacific island taxa to occur sympatrically with their closest relative (Mayr & Diamond

2001, Andersen et al. 2015, Cowles & Uy 2019, Manthey et al. 2020; but see DeCicco et al. 2024). To better understand why intra-archipelago speciation is so rare in South Pacific birds, it is important to study taxa that come into secondary contact with previously isolated congeners, as has been done with the well-studied hybrid zones found on continents (Toews et al. 2016a, Brelsford et al. 2017, Wang et al. 2020).

Natural invasions between allopatric island lineages are rarely observed. As such, there are few documented cases of traditional hybrid zones forming on islands between avian lineages in secondary contact (Graves 2015, Sardell & Uy 2016, Shogren et al. 2024). Instead, hybridization in island birds often results in hybrid populations, an observation first noted through careful examination of museum specimens collected across Melanesia (Mayr 1932a, 1932b, 1938, 1942; Mayr & Diamond 2001). Recent genetic studies have further supported this phenomenon in islands and 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, Andersson et al. 2024). This is because islands—especially smaller ones—are unlikely to be large enough to host the wide hybrid zones of dispersive taxa (Barton & Hewitt 1985, McEntee et al. 2020) and lack consistent parental input necessary for maintaining early generation hybrids (Kinsey 1937, Mayr 1942, Diamond 1977, Kissel and Barraclough 2010). Furthermore, on small islands adjacent to large islands and continents, the greater number of potential migrants originating from the larger land mass can lead to genetic swamping and admixture in the smaller island with smaller census populations and fewer potential migrants (see MacArthur & Wilson 1963, 1967; Gyllenhaal et al. 2020). The prevalence of hybrid populations, as opposed to hybrid zones, makes the empirical cases of ongoing secondary contact between island birds of particular interest. These "moments of truth" where reproductive isolation is tested not only provide exceptional insight into the speciation process, but potentially into island hybrid populations form (Mayr 1942, Shogren et al. 2024).

Bird lineages across the Indo-Pacific are often described as being ‘polytypic,’ meaning they are species with many phenotypically distinct subspecies, making them well-suited for studying the early stages of speciation. Among these lineages, *Pachycephala* whistlers are one of the most widespread and strikingly diverse geographic radiations in the Indo-Pacific (Galbraith 1956). Ernst Mayr used this ‘great speciator’ lineage to shape our early understanding of allopatric speciation (Mayr 1932a, 1942, 1963; Mayr & Provine 1980). While most insular populations of *Pachycephala* are allopatric, several instances of secondary contact between divergent lineages have been documented, resulting in outcomes ranging from sympatry to apparent hybrid populations (Mayr 1932a, Mayr 1932b, Mayr & Diamond 2001). This presents a rare opportunity to study dynamics of secondary contact of a species that makes up part of an assemblage that is predominantly characterized by strict allopatry.

The Fiji Whistler (*Pachycephala vitiensis*) is a monophyletic lineage comprising 10 named taxa (Andersen et al. 2014; Jønsson et al. 2014) and is part of the broader Golden Whistler species complex (Galbraith 1956, 1967). Most taxa are single-island endemics that are distinct in appearance; yet, their distinctiveness consists of variations on a theme. For example, most male golden whistlers have dark backs and yellow underparts, but populations vary in combinations of throat color (white or yellow), the presence or absence of a black breast band, and other minor plumage details. Thus, three main population types are found: 1) white-throated with a black breast band, 2) yellow-throated with a black band, and 3) yellow-throated with no band (Fig. 1a). Typically, one of these phenotypes occurs across an entire archipelago (e.g., *P. citreogaster* in the Bismarck Archipelago and *P. chlorura* in Vanuatu are white-throated); however, all three plumage types are present within Fiji.

Mayr hypothesized that this variation in the Fiji Whistler was driven by two waves of colonization into Fiji: first by a yellow-throated, unbanded lineage, followed by a newly expanding white-throated, banded lineage. He proposed that these lineages subsequently formed a series of hybrid populations, featuring yellow throats from the first lineage and black bands from the second. This hypothesis contrasts with a parsimonious model of steppingstone colonization, but its complexity is supported by the existence of a contact zone on Vanua Levu between two yellow-throated taxa—one banded and one unbanded (Fig. 1a). Mayr's hypothesis played a significant role in some of his influential works (e.g., Mayr 1942, Mayr 1963), with the evidence of failed secondary contact supporting his conclusion that allopatric lineages like these do not constitute biological species (Mayr 1932a).

Here, we use genomic data to test Mayr's hypotheses: 1) that white-throated taxa represent an geographically expanding clade, and 2) that all yellow-throated banded populations are hybrids between unbanded and white-throated taxa. We hypothesize that Fiji's whistlers have a stepping-stone colonization history, with yellow-throated taxa nested within white-throated taxa in the phylogenetic tree. Additionally, while we expect gene flow will play a prominent role in this archipelago radiation, we do not anticipate it manifesting as admixture between deeply divergent taxa. In short, we hypothesize a simpler history than Mayr envisioned, but still one characterized by weak species boundaries. Furthermore, we use the known hybrid zone between two phenotypically divergent taxa on Vanua Levu (Fig. 1a) to examine the genomic landscape of divergence. Given the apparent widespread admixture occurring in this contact zone, we hypothesize that divergence will be limited to a few genomic regions (Barton 1983, Flaxman et al. 2014). We address these questions using two reduced-representation genomic datasets and a new draft reference genome.

Methods

Sampling and DNA Extraction

We collected genetic data from all major phenotypes across the Fijian archipelago, with a particular focus on the contact zone in Vanua Levu (Fig. 1). After excluding samples with insufficient reads or signs of relatedness (evidenced by pairs of individuals showing as disproportionately divergent in principal component space only when together), we sampled 79 individuals of the genus *Pachycephala* in our primary RADseq (restriction site-associated DNA sequencing) dataset, 76 of which comprised our core sampling of *P. vitiensis*. All individuals were represented by tissues with vouchered specimens (Table S1). We sampled 63 yellow-throated Fijian taxa (formerly *Pachycephala graeffii*): seven from Ovalau (*P. v. optata*), four from Viti Levu (*P. v. graeffii*), 24 from Vanua Levu (*P. v. aurantiiventris* and *ambigua*), 13 from Rabi (*P. v. ambigua*), seven from Kioa (*P. v. ambigua*), and seven from Taveuni (*P. v. torquata*). For white-throated taxa, we sampled five from Kadavu (*P. v. kandavensis*), eight from Ogea Levu (*P. v. lauana*), one from Vuagava (*P. v. lauana*), and three from Nendo (Solomon Islands; *P. vanikorensis ornata*). We lacked three single-island subspecific populations of *P. vitiensis*: the yellow-throated *P. v. bella* and *P. v. koroana* from Vatu Vara and Koro, respectively, and the white-throated *P. v. vitiensis* from Gau. In addition to our primary RAD dataset, we used one sample of *P. v. kandavensis* (KU Birds 117379) to generate a nuclear reference genome assembly using linked-read sequencing on the 10X chromium platform. To assess the timing of divergence of our focal clade, we also compiled a dataset of ultraconserved elements (UCEs; Faircloth et al. 2012) from 36 individuals across the *P. pectoralis* complex, which includes *P.*

vitiensis Table S1. This UCE dataset expands Clade A from Brady et al. (2022) and includes 24 newly sequenced samples along with 12 samples sequenced from Brady et al. (2022). Four of these samples were derived from museum specimen toepads (two each from *P. jacquinoti* and *P. flavifrons*), while the rest came from specimen-vouchered tissues (Table S1).

Reduced-representation Bioinformatics

We used Stacks v2.4.1 (Rochette et al. 2019) to call single nucleotide polymorphisms (SNPs) for our focal RADseq dataset, and these outputs were used for downstream analyses. We used the *process_radtags* module in single-end mode to demultiplex reads by individual, which were then aligned to our reference genome using the *mem* algorithm of BWA v0.7.17 (Li & Durbin 2009). We used the Stacks module *gstacks* to assemble RAD loci from these alignments and to call SNPs. We generated multiple datasets using *gstacks* for different analyses and used the *gstacks* output to generate input files using the *populations* module. Unless otherwise stated, we performed these analyses with a 75% complete SNP matrix (i.e., SNPs present in 75% of individuals were included), which is a common cutoff for this type of data. We processed UCEs using the phyluce v1.7.0 pipeline (Faircloth 2015), following the specific steps outlined in Brady et al. (2022).

Population structure

To confirm the genetic relationships among populations, we used several methods to examine population structure at all levels using RAD data, but with a particular focus on yellow-throated *P. vitiensis*. First, we calculated Weir and Cockerham's (1984) F_{ST} between each sampling region with more than one individual (island or sub-region, where relevant) using a custom wrapper around vcftools v0.1.15 (Danecek et al. 2011). Although Weir and Cockerham's estimator can be biased slightly for low sample sizes at higher levels of divergence, this was not a concern for our focal comparisons (Willing et al. 2012). This script was initially developed for Mapel et al. (2021), and an updated version is available on GitHub: https://github.com/ethangyllenhaal/FijiPachyRad. We conducted the analysis separately for loci assigned to autosomes and the Z chromosome. Second, we performed principal component analyses (PCAs) using the *glPca* module of adegenet v2.1.1 (Jombart 2008) in R v3.6.1 (R Core Team, 2019), which uses imputation to address missing data. The input for PCA was generated using the R package vcfR v1.8.0 (Knaus and Grünwald, 2017). Finally, we assessed admixture proportions of yellow-throated *P. vitiensis* using sNMF (Frichot et al. 2014) in the R package LEA v2.2.0 (Frichot & François, 2015). Input for sNMF excluded uninformative singletons (i.e., minor allele count <2; Linck & Battey 2019). The alpha (normalization) parameter was set to 10 after exploratory runs to minimize the cross-entropy criterion, although results were similar across different values. The result with the minimum cross-entropy criterion after 50 iterations per k value was selected for plotting.

Phylogenetic trees and networks

To test Mayr's hypothesis of colonization history, we generated a phylogeny of our RAD data using a concatenated maximum likelihood approach in IQ-TREE v2.8.3 (Minh et al. 2020), treating heterozygotes as equal likelihoods of either nucleotide. We used ModelFinder (Kalyaanamoorthy et al. 2017) for model selection and assessed support with 1000 ultrafast bootstraps (Hoang et al. 2018). The analysis was based on individual-level, phylip-formatted data from a 90% complete matrix. Two samples were found to be problematic (showing unexpected placement based on other analyses, thereby reducing node support values for intervening samples) and were removed from subsequent sequence-based analyses. We also estimated a species tree using SVDQuartets in PAUP* v4.0a169 (Chifman & Kubatko 2014), with 100 bootstraps and evaluating 100,000 quartets, using the same 90% complete matrix as the IQ-TREE analysis and grouping samples by sampling region (Fig. 1). To estimate the age of our focal radiation, we conducted divergence time dating in BEAST v2.6.7 (Bouckaert et al., 2019; Appendix 1).

To account for reticulation and major introgression events, we constructed a phylogenetic network (specifically a Neighbor-net) using SplitsTree v4.14.6 (Hudson 1998) and a population tree with inferred admixture events using TreeMix v1.13 (Pickrell & Pritchard 2012). Both analyses used a 90% complete SNP matrix to minimize the effects of missing data. Input for SplitsTree was based on Nei's D genetic distances calculated with StAMPP v1.6.3 (Nei 1972, Pembleton et al. 2013). For TreeMix, we iteratively added migration edges until the last meaningful edge was added that increased the likelihood by more than two (equivalent to a change of the Akaike Information Criterion of four, which is a common threshold for model selection).

Tests for gene flow

To test Mayr's hypothesis that banded taxa are of hybrid origin, we performed statistical tests for gene flow using ABBA/BABA tests in Dsuite v0.4r38 (Malinsky et al. 2021), with *P. vanikorensis* as an outgroup and a 90% complete VCF as input. We calculated the D statistic (Durand et al. 2011) and assessed its significance, along with the f_4 estimate of admixture proportion (Patterson et al. 2012). Additionally, we used the f_{branch} function to infer the timing of gene flow (Malinski et al. 2018). To address multiple comparisons, we employed two approaches, as described in Appendix 1 (Supplemental Methods; Correcting for multiple tests for gene flow). We opted for this approach over demographic modeling due to the complexity of the contact zone and uncertainties regarding the exact relationships among populations, particularly those that were not sampled.

Genome scans

Modern research on speciation has highlighted the importance of genomic architecture in trait divergence and its influence on the outcomes of secondary contact. To assess the landscape of divergence, we estimated Weir and Cockerham's (1984) weighted F_{ST} in 10 kbp windows across the genome using vcftools v0.1.15 (Danecek et al. 2011), and plotted the results using the R package qqman v0.1.8 (Turner 2018). In most cases, this windowed value represents the mean F_{ST} of a single RAD locus; windows without SNPs were excluded. We took two approaches to assign comparisons: by geographic locality and phenotype. The geographic approach represented a standard inter-taxon F_{ST} outlier comparison, whereas the phenotypic approach was similar to a genome-wide association study (GWAS). Phenotypic indices were estimated by MJA and included measures for the extent of yellow in the lores and the presence of black on the breast in yellow-throated *P. vitiensis*. Specifically, the band extent was binned into four categories (absent, hooked, dirty, and complete; scored 0–3, respectively), while the lores were binned into three categories (black, minimally yellow, and completely yellow; scored 0–2, respectively). We created a cumulative hybrid index by adding the band score to two minus the lore score (to ensure "pure" unbanded birds had an index of 0). We also examined putative functional genes based on our annotations (Supplemental Methods) for genomic windows with F_{ST} values greater than 0.5 in the eastern vs western Vanua Levu comparison, including a region on the Z chromosome bounded by values >0.5.

Results

Population structure

We identified hierarchical genetic structure across this archipelago radiation, with notably less pronounced structure among yellow-throated taxa compared to white-throated taxa (Fig. S1a). Within the yellow-throated taxa, there was significant population structure corresponding to geographic location and the extent of dark plumage in each population (Figs. 2b, S1b; Table S3). In principal component space, most intermediate and banded taxa were similar along PC1, while populations from Rabi and Taveuni separated on PC2 (Fig. S1b). In population assignment analyses, admixture fractions were primarily attributed to a single ancestral population, though consistently high levels of mixed ancestry were inferred for eastern Vanua Levu (Fig. 2b). Genome-wide F_{ST} estimates across Vanua Levu were associated with plumage coloration; specifically, F_{ST} increased from western to eastern Vanua Levu and was even higher for darker island populations on Kioa, Rabi, and Taveuni (Table S3). In general, F_{ST} values were higher on the Z-chromosome compared to autosomes, except in the comparison between Kioa and eastern Vanua Levu. Despite its intermediate plumage, the Ovalau population was distinct in all population structure analyses.

Phylogenetic trees and networks

Our phylogeny inferred using IQ-TREE indicated that the white-throated Lau populations are sister to the rest of *P. vitiensis*, with the population on Kadavu being sister to all yellow-throated taxa (Fig. 2c, Fig. S2). This means that the white-throated taxa did not form a monophyletic group and had notably older branches than the yellow-throated taxa. Within the yellow-throated populations, individuals from Ovalau were sister to all other yellow-throated populations, while Taveuni was moderately supported (bs= 90) as sister to the remaining yellow-throated clade. Support values across the rest of the tree were low, and many populations that clustered in population structure analyses were not monophyletic.

Our phylogenetic network showed high levels of reticulation among yellow-throated taxa despite some population-specific clustering, consistent with the low support values (Fig. S3). The population trees from TreeMix produced a similar topology to our concatenated analyses when no migration edges were included (Fig. 2d, S4). However, when migration was modeled, a significant migration edge was inferred between Ovalau and an ancestral taxon (admixture fraction=0.376; $p < 0.0001$), which resulted in a change in Ovalau's placement within the tree to being sister to the southern Viti Levu population, nested within the clade of populations with weaker breast bands (Fig. 2d). When considering two migration edges, a second, less significant ($p = 0.0067$) edge was inferred between an ancestral Kadavu population and the ancestor of the Viti Levu clade (Fig. 2d). The phylogenetic network showed high levels of reticulation for the Ovalau population without a direct connection to other sampled populations, such as Kadavu (Fig. S3). Our species tree analysis using SVDQuartets produced similar results but was very sensitive to the inclusion of the Ovalau population (Fig. S5). While several nodes had decent support without Ovalau (often stronger than in the IQ-TREE analysis), including the putatively admixed Ovalau population led to high topological uncertainty. Our UCE time tree (Fig. 1b, Fig. S6) suggested that the crown age of Fijian whistlers is approximately 0.75–2.58 Ma (mean 1.49 Ma), which is younger on average than the Solomon Islands clade (*P. orioloides*; 0.89–3.28 Ma; mean 2.11 Ma). The mean ages of island-specific taxa in Fiji range from 0.66–0.96 Ma.

Tests for gene flow

Tests for gene flow within the group provided evidence of widespread gene flow among yellow-throated taxa, but little admixture between banded and white-throated populations.

Significant tests ($p < 0.05$) between adjacent populations are summarized in Figure 3a, with exact tests detailed in Table S4 and S5, and f_{branch} results in Figure S7. In summary, high levels of gene flow (f_4 admixture fraction > 0.2) were inferred between 1) Kioa and western and central Vanua Levu, 2) Rabi and points west, 3) Taveuni and both Rabi and Kioa, and 4) Ovalau and Viti Levu. The latter pairing was highly significant, likely due to the admixed nature of Ovalau (as inferred by TreeMix), which resulted in unusual topologies being tested. All of these gene flow events were significant after accounting for multiple comparisons, except for gene flow between Rabi and points west (harmonic p=0.068) and between eastern Vanua Levu and the rest of Vanua Levu (harmonic p=0.053). However, the latter is supported by phenotypic evidence. Low levels of gene flow were inferred between Viti Levu and Kadavu, and between Ogea Levu and several other populations (Viti Levu, central Vanua Levu, Rabi, and Kioa). Although these results did not hold up to corrections for multiple comparisons, gene flow between Viti Levu and Kadavu (or a related population) was supported by TreeMix (Fig. 2d).

Genome scans

Our examination of the genomic architecture of divergence revealed one consistent peak (i.e., multiple consecutive windows with high F_{ST}) in the comparison between eastern and western Vanua Levu, which differentiated the darker and yellower populations against a background of low overall divergence. This peak resided on the Z chromosome and was most prominent between populations with the most similar genome-wide F_{ST} but differing breast band patterns (i.e., eastern vs. western Vanua Levu; Fig. 4). We also identified several other peaks between Taveuni and other populations, including a broader peak on the Z chromosome (Fig. S8).

Our assessment of potential causal loci found two genes previously linked to plumage or pigmentation in past studies. One of these genes, TRPM7, belongs to the same family as TRPM1, which has been associated with feather pigmentation in domesticated chickens (Gu et al. 2024). More notably, PCSK5 has been directly correlated with variation in melanin levels in wild birds and is involved in melanin production pathways (San-Jose et al. 2017). Additionally, several genes associated with other phenotypes, such as fat deposition (NR2F2; Zhu et al. 2021) and stress responses (MCTP1; Taff et al. 2019), were also found within this large region.

Discussion

The genus *Pachycephala*, particularly those in Fiji, played a significant role in shaping Ernst Mayr's views on allopatric speciation during the Modern Synthesis (Mayr 1932a, Mayr 1932b, 1942). Mayr interpreted phenotypic evidence as indicating multiple instances of hybrid populations following phenotypic divergence in allopatry, suggesting that these lineages failed to achieve reproductive isolation despite displaying plumage differences comparable to some continental sympatric species within the genus (e.g., *P. soror* and *P. schlegelii*, *P. pectoralis* and *P. melanura*). Our study supports the general idea that these birds did not fully speciate while in allopatry, but we did not find support for Mayr's specific hypotheses of 1) a monophyletic white-throated lineage and 2) universal admixture in banded populations (Fig. 3b). Instead, we propose a hypothesis emphasizing rapid plumage evolution in geographic allopatry as the species colonized Fiji, resulting in greater diversity in male plumage compared to the older radiation in the Solomon Islands (*P. orioloides*; Fig. 1b, 3c). A subsequent wave of movement—potentially facilitated by glacial cycles that reduced distances between islands—led to secondary contact, which in turn caused the formation of a hybrid population on Ovalau (Fig. 2d) and a series of intermediate populations across Vanua Levu (Fig. 2b, Table S3).

History of colonization and plumage divergence

Our results support two key aspects of Mayr's hypothesis: dynamic waves of colonization and the lack of reproductive isolation between allopatric lineages (Mayr 1932a, 1942). However, the specific colonization dynamics we inferred differed significantly from Mayr's interpretation. First, we found no evidence of recent contact between "old" and "new" phenotypes, as indicated by the monophyly of yellow-throated taxa and the non-monophyly of white-throated taxa (Fig. 2c). Additionally, we found no evidence that all banded, yellow-throated populations—particularly those on Taveuni—shared an excess of alleles with white-throated lineages (Fig. 2d, 3a, S4).

We propose an alternative model for the colonization of Fiji whistler. Initially, a white-throated lineage colonized the Fijian archipelago, followed by the evolution of yellow-throated phenotypes and a second wave of dispersal among predominantly yellow-throated populations, which led to the two admixture scenarios described above (Fig. 3c). The phylogenetically nested nature of yellow-throated Fijian populations within a clade of white-throated Kadavu and Lau populations (Fig. 2c) suggests an initially white-throated, dispersive lineage colonized the isolated islands of Fiji (as in DeRaad et al. 2024). Second, the loss of the white throat in northern and western Fijian populations suggests a single evolutionary origin of the yellow-throated phenotype during the northward colonization of the archipelago. Third, the nested nature of unbanded populations suggests the loss of the black breast band, while outlying islands (e.g., Koro, Taveuni, and Rabi) retained the banded phenotype.

Finally, we find evidence for a recent wave of admixture, possibly driven by glacial cycles. The first example is increased gene flow from Rabi and Kioa to the Natewa Peninsula of Vanua Levu—which is relatively isolated from the rest of the island due to its long, narrow base—resulting in phenotypic intermediates across the Peninsula. Glacial cycles may also have led to the colonization of Viti Levu by this clean-breasted form, potentially filling an empty niche or displacing a white-throated ghost population related to the birds on Kadavu, which is supported by a detectable signal of gene flow (Figs. 2d and 3a). 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 the continued separation during glacial periods (Fig. 2, Table S3). Finally, during this colonization wave or during glacial maxima, the island of Ovalau was partially genetically swamped by the new clean-breasted lineage that had colonized Viti Levu. The relatively distinct nature of Ovalau, an island that was fully connected to Viti Levu during glacial maxima, is consistent with admixture occurring during a recent glacial cycle; extended contact would likely have completely eroded the genetic and phenotypic signatures of its admixed origin. Indeed, once gene flow is accounted for, Ovalau is nested within the Viti Levu populations (Fig. 2d). Finally, estimating the timing of these events in a phylogenetic context is complicated by intraspecific divergence (Fig. 1b) and confounding gene flow (Fig. 3a). However, our proposed historical model is supported by multiple lines of genetic evidence and offers a parsimonious explanation regarding the number of colonization waves and extent of gene flow between extant taxa.

This colonization pattern differs from another taxon with a similar distribution, the wattled honeyeaters (*Foulehaio*). In this case, older diverging lineages occupy the larger islands of Fiji, while more isolated islands in the Lau, Tongan, and Samoan archipelagos host recently diverged populations with evidence of long-distance gene flow among them (Mapel et al. 2021). In contrast, *Pachycephala* exhibits the opposite pattern, with populations in Lau, Tonga, and Samoa being sister to those on the larger Fijian islands (Fig. 1b, 2c). Interestingly, the genetic and phenotypic affiliation between the Natewa Peninsula and Taveuni aligns with the restricted distribution of *Lamprolia* silktails (Aves: Rhipiduridae)—a deeply divergent relictual lineage—found only in those two regions (Irestedt et al. 2008, Andersen et al. 2017). These observations highlight that while we can make some predictions about how recently diverged, allopatric populations may interact upon secondary contact, colonization history can be highly idiosyncratic even among co-distributed lineages.

Secondary contact of allopatric lineages

Our dataset included two instances of secondary contact: a contact zone on Vanua Levu and a genomically confirmed hybrid population on Ovalau. This hybrid population on Ovalau is particularly intriguing, as we are confident that one parent is the unbanded, yellow-throated population from Viti Levu, while the other appears to be an unsampled or ghost taxon that likely originally inhabited Ovalau before this merger (Fig. 2d, 3a, S5). The phenotype and genetic affinities of this ghost taxon are unclear, but it may be related to extant populations found on Bega, Koro, or Gau islands, or it could represent a subspecies that has now been genetically swamped. This finding supports the idea that small islands are more conducive to forming hybrid populations rather than traditional hybrid zones (Mayr 1932b, Mayr 1938, Nietlisbach et al. 2013, Lavretsky et al. 2015, Colella et al. 2021, Andersen et al. 2021, Andersson et al. 2024). The lack of consistent parental input may significantly impact the sorting of genomic variation, and these novel combinations could lead to the formation of hybrid species if reproductive isolation evolves (Ottenburghs 2018). Additionally, this highlights the potential importance of certain island populations as genetic repositories of now-extinct populations. This situation also poses a taxonomic challenge, as systematists must consider how to treat these genetically distinct populations taxonomically, especially given their restricted range. Indeed, if Ovalau's hybrid population retains genetic diversity from what was once a subspecies-level taxon, it underscores the critical importance of ongoing efforts to conserve this island's forests (Vula 2022). Well-sampled genomic studies will be essential for documenting hybrid populations like these, especially for cryptic taxa (e.g., Andersen et al. 2021).

In addition to testing classic hypotheses of secondary contact in allopatric lineages, our genomic dataset allowed us to identify a single multi-locus divergent genomic region that appears to drive plumage divergence on Vanua Levu (Fig. 4). Although one gene within this region (PCSK5) has mechanistic ties to plumage divergence (San-Jose et al. 2017), it is part of a larger region containing genes involved in other pathways. While it is tempting to attribute causality to PCSK5, we believe further validation is necessary to rule out other explanations, such as genetic hitchhiking. Indeed, even if PCSK5 is causal, the phenotype itself may not have evolved under strong selection but could instead be a result of hitchhiking with a linked gene under selection or genetic drift (Gould & Lewinton 1979, Barton 2000). Furthermore, while reduced-representation sequencing is useful for identifying larger regions and providing an overall view of divergence (Toews et al. 2016b, Irwin et al. 2018, Musher et al. 2023), its low resolution limits our ability to conclusively rule out smaller causal regions, especially given the fragmented nature of our non-scaffolded reference genome. However, the resolution is sufficient to demonstrate that a notable portion of genomic divergence is concentrated within a relatively small region across the focal contact zone (Fig. 4).

Our finding of a single chromosomal region underlying plumage divergence is significant for understanding geographic radiations. Even if selective forces shape this divergence, it is unlikely to create robust species boundaries, as polygenic adaptation is more effective at promoting cohesion during secondary contact (Barton 1983, Barton & Hewitt 1985, Flaxman et al. 2014). If this region is indeed broadly responsible for the similar plumage diversity observed in other whistlers, it may suggest a model in which great speciators evolve significant plumage divergence despite minimal intrinsic reproductive isolation or ecological specialization.

Combined with our findings of admixture between divergent lineages, this framework supports the hypothesis that geographic radiations—particularly those within a single archipelago—should not be assumed to be reproductively isolated based solely on plumage differences. Even if intrinsic selection against hybrids evolves, without strong assortative mating, it may still lead to reproductive interference (i.e., hybridization without viable offspring; Mikkelsen & Irwin 2021, Irwin & Schluter 2022). This scenario could provide an additional mechanism driving the evolution of reduced dispersal over time in island birds, which may, in turn, help resolve the 'paradox' of the great speciator (Diamond et al. 1976, Moyle et al. 2009, Wright et al. 2016, DeRaad et al. 2024). Although Mayr's specific hypothesis regarding the colonization history of Fiji was incorrect, our findings support his broader conclusion that allopatric island lineages cannot necessarily be considered distinct biological species. Instead, great speciators may possess genomes that are only as resistant to admixture as the geographic barriers between them.

Conclusion

Our work revisited a system that had a disproportionately large influence on Mayr's contributions to the Modern Synthesis and the Biological Species Concept (Mayr 1942). Like

Mayr, we concluded that reproductive isolation among these whistlers is likely weak, but that they are important for understanding the evolution of geographic radiations and contain many important elements of speciation theory. Our findings also align with recent research on the genomic architecture of speciation, showing that high levels of introgression across Vanua Levu occur with few genomic regions associated with the differences among populations (Barton 1983, Flaxman et al. 2014).

We also provided further evidence that small islands can foster the formation of hybrid lineages (although not universally), and that genomic data can uncover potential reservoirs of unrecognized genetic diversity. Additionally, as increasingly demonstrated by recent studies, geographic variation in plumage coloration can evolve rapidly, with two major transitions in plumage phenotype occurring in a relatively short span of time (approximately 500,000 years). Finally, our study highlights the value of historic and modern collections in reexamining long-standing hypotheses about species formation and diversification.

Acknowledgements

We are indebted to the staff and curators at the South Pacific Regional Herbarium, University of the South Pacific, Suva, Fiji (Marika Tuiwawa and Tokasaya Cakacaka), the Fiji Department of Forestry (Sanivalati Vido), the Biosecurity Authority of Fiji (Joeli Vakabua), Mika Bolakania, and Dick Watling for their assistance, permission, and friendship in Fiji. We are grateful to the following people and institutions for loaning new samples necessary for this project: Mark Robbins (University of Kansas Biodiversity Institute), Paul Sweet (American Museum of Natural History), and Sharon Birks (University of Washington Burke Museum, USA). We are also grateful to the following people for loaning samples originally sequenced in Brady et al. 2022: Jack Dumbacher and Maureen (Moe) Flannery (California Academy of Science), Alex Drew

(Australian National Wildlife Collection), and Robb Brumfield (Museum of Natural Science at Louisiana State University). We thank Jenna McCullough, Nick Vinciguerra, David Tan, and Liz Solis, whose comments helped improve the manuscript. We would like to thank the UNM Center for Advanced Research Computing, supported in part by the National Science Foundation (NSF), for providing the high-performance computing resources used in this work. We thank the KU Genome Sequencing Core (supported by National Institutes of Health grant 5P20GM103638 to E.A. Lundquist) for access to lab equipment and services. We gratefully acknowledge funding from the NSF's Graduate Research Fellowship (DGE-1650114) to EFG and NSF awards to RGM (DEB-1557053) and MJA (DEB-1557051, DEB-2112467).

Data Accessibility Statement

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

Supplementary Material

Supplementary files including two sampling tables and an appendix of supplemental figures, methods, and results are available online.

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Figure Captions

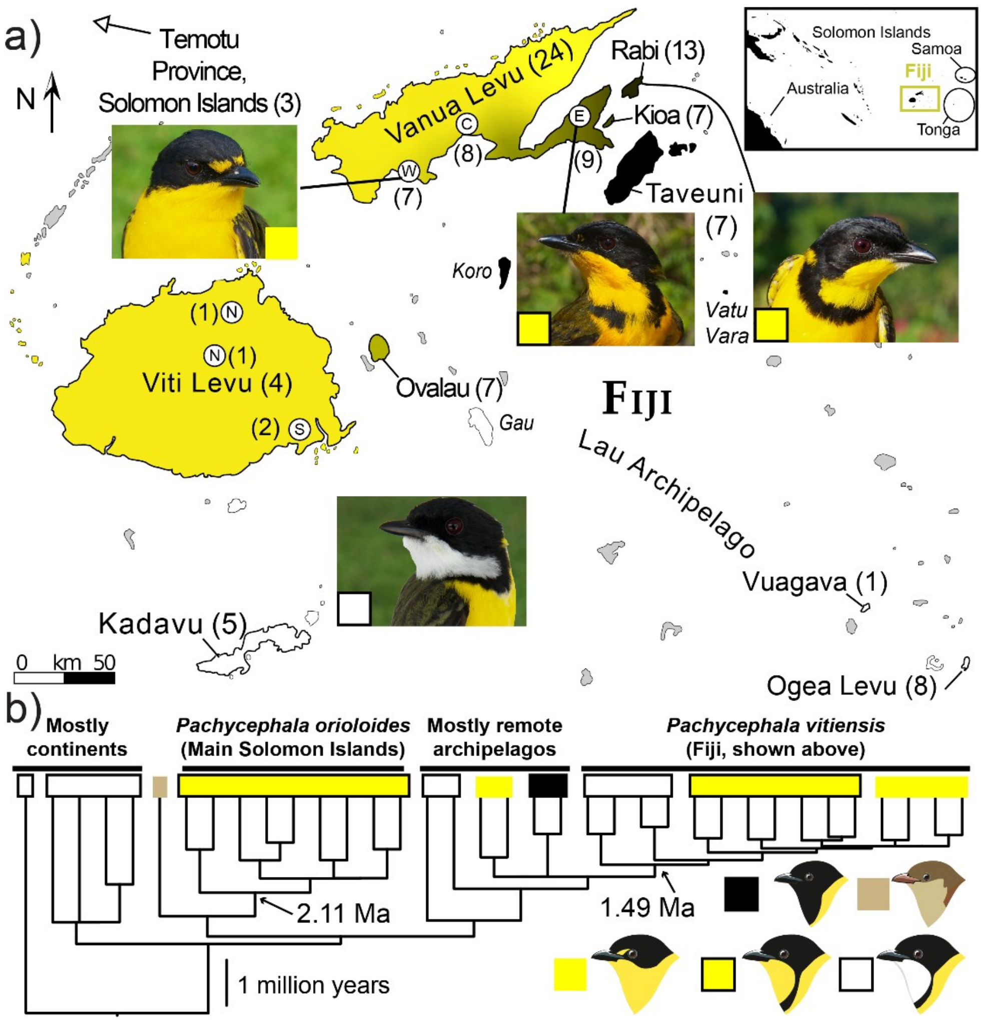

Figure 1: Sampling map and examples of our focal phenotypes. a) Sampled islands are labeled by name with number of individuals sampled in parentheses. Unsampled islands with distinct taxa are labeled in italics. Island color corresponds to phenotype; gray islands lack populations of whistlers. White 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 (shades of olive) represent approximate extent of black breast band. Photos of archetypical individuals are shown, with a box corresponding to the color scheme used in panel B. Sampling points (white circles) on large islands are shown, with locality names in the center. Inset depicts the geographic location of Fiji. b) Phylogeny inferred in BEAST from ultraconserved elements, including additional whistler species from the *P. pectoralis* species complex used for estimating divergence dates. Tips have boxes corresponding to male plumage patterns, with cartoon examples of phenotypes in the bottom right corner. Tip names can be found in Fig. S6. Mean divergence times are labeled for the crown age of *P. orioloides* (2.11 Ma) and *P. vitiensis* (1.49 Ma). Artwork by Jenna McCullough, based on artwork from Birds of the World (Billerman et al. 2020).

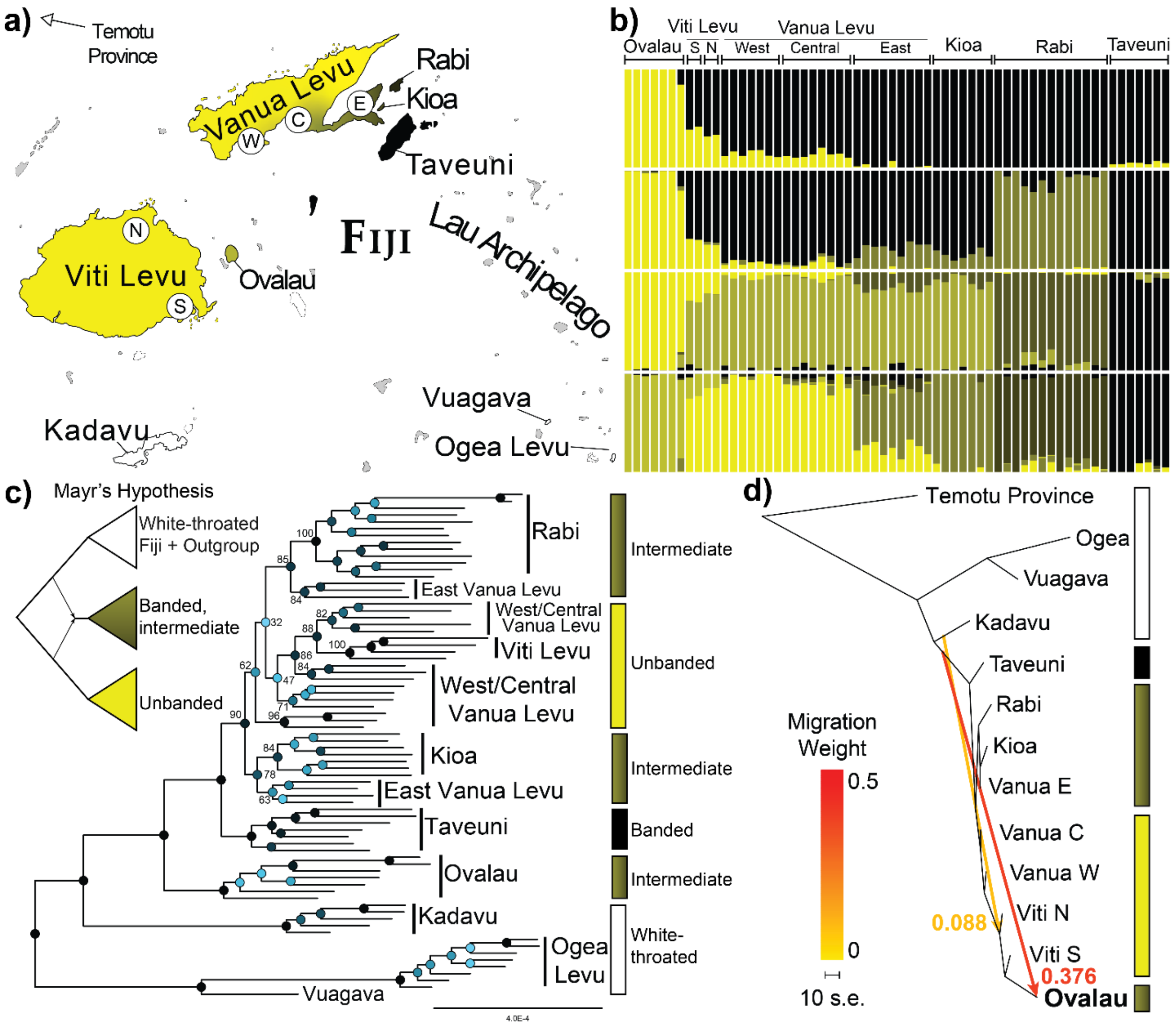

Figure 2: Phylogenetics and population structure of *Pachycephala vitiensis*. a) Map of focal sampling localities in Fiji. North, South, East, West, and Central are abbreviated to N, S, E, W, and C, respectively. b) Ancestry plots from sNMF analyses of yellow-throated taxa for K values of 2–5, colored on a yellow-black gradient corresponding to the relative mean band extent in the population (more extensive bands in blacker clusters). The value of K that minimized the cross-entropy criterion is 3. c) 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. Inset represents a phylogenetic network based on Mayr's hypothesis, notably including a monophyletic white-throated lineage. d) TreeMix population tree with two migration edges, with labels corresponding to the weight of the edge (see Figure S3 for results using zero and one migration edges).

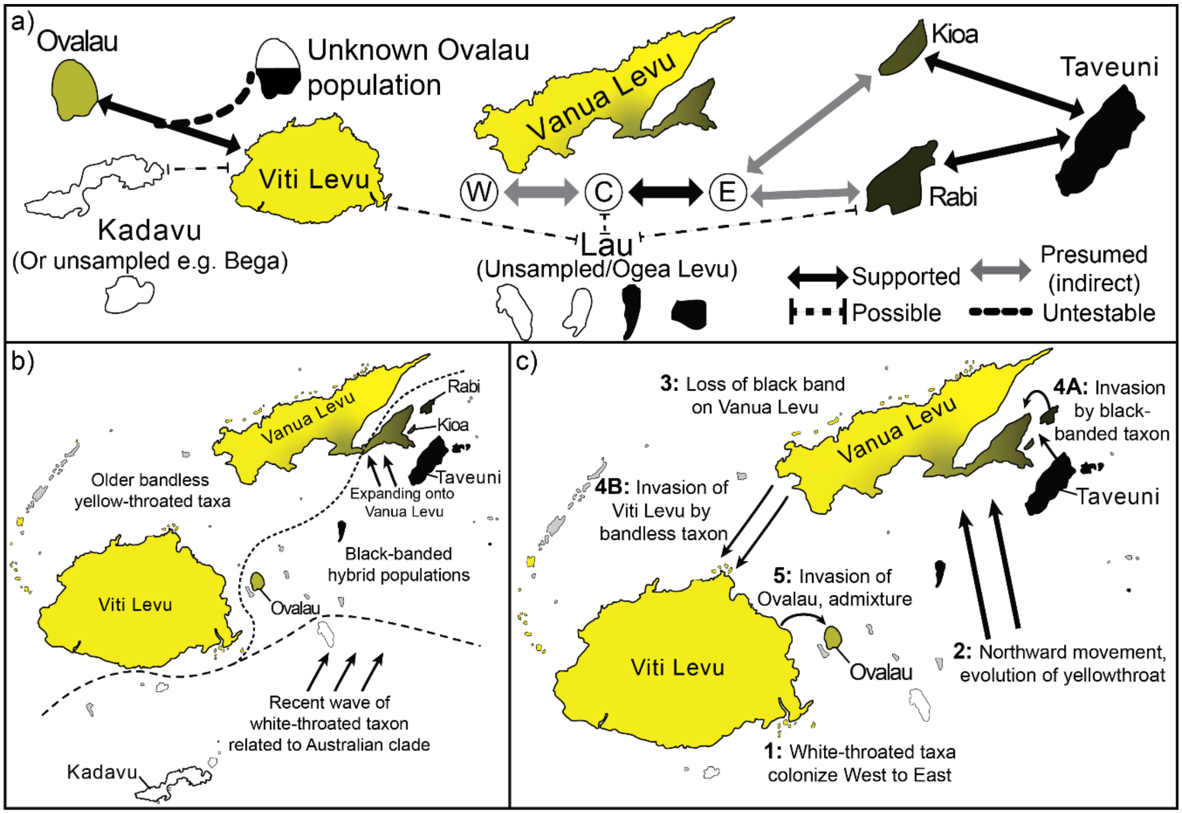

Figure 3: Inferences of gene flow and hypotheses of colonization history in *Pachycephala vitiensis*. a) Sampled populations (represented by their respective islands' shape and phenotype) with arrows corresponding to admixture fraction as estimated by the f4 ratio. The "possible" lines (narrow and dashed with flat ends) denote edges with significant gene flow for some tests but that did not stand up to multiple comparisons. "Presumed" edges (solid gray arrows) 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. Diagrams of (b) Mayr's and (c) our hypothesized colonization history of whistlers in Fiji.

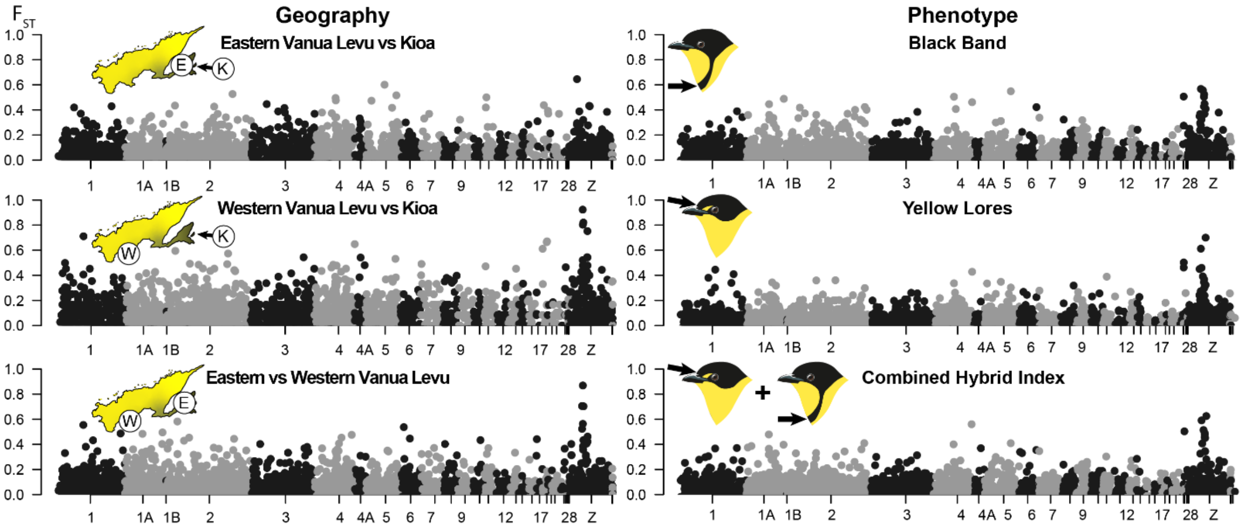

Figure 4: Manhattan plot of F_{ST} across the whole genome (based on synteny mapping with Zebra Finch *Taeniopygia guttata*). The left column compares by population (with East Vanua Levu and Kioa sharing similar phenotypes), while the right column compares by phenotype. For the right column, the values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