Complex mosaic of sexual dichromatism and monochromatism in Pacific robins results from both gains and losses of elaborate coloration

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ABSTRACT

Pacific robins exhibit one of the most complex range-wide mosaics of sexual dichromatism and monochromatism. The evolutionary origins of this geographic mosaic remain poorly understood despite long-standing interest from ornithologists, and its influential role in the development of Ernst Mayr's theories on speciation and the Biological Species Concept. One factor limiting our understanding of the evolution of sexual plumage variation in Pacific robins is a lack of well-resolved taxon boundaries and phylogenetic relationships in the group. Here, we use primarily historical museum specimens to obtain dense sampling of mtDNA, nuclear DNA, plumage color and morphometrics from all named taxa in the radiation in order to infer taxon boundaries and relationships. We use these data to test hypotheses about colonization history, plumage evolution and reduced island dichromatism. Our data show that the Pacific robin radiation comprises four distinct lineages, which warrant recognition as separate species – the previously recognized Norfolk robin *P. multicolor* and red-capped robin *P. goodenovii*, and two new species we propose to name: "Solomon robin" *P. polymorpha* Mayr, 1934 for the populations on Solomon and Bougainville Islands, and "Mayr's robin" *P. pusilla* Peale, 1848 (in honor of Ernst Mayr's detailed work on the southwest Pacific robins) for the populations on Vanuatu, Fiji and Samoa. Our data suggest that the common ancestor of the entire Pacific robin radiation was most likely sexually dichromatic and that the radiation-wide mosaic of sexual plumage color arose via repeated losses of elaborate plumage in males and gains of elaborate plumage in females on separate islands.

Keywords: sexual dichromatism, island speciation, southwest Pacific, plumage color, spectrophotometry, ancestral state reconstruction, reduced island dichromatism

INTRODUCTION

Sexual dichromatism evolves via an interplay between natural and sexual selection (Bennett and Owens 2002, Heinsohn et al. 2005, Matysioková et al. 2017, Shultz and Burns 2017). Island populations are known for being less colorful, less sexually dimorphic and producing less complex song compared to their mainland congeners (Omland 1997, Grant 2001, Badyaev and Hill 2003, Baker et al. 2006, Roulin and Salamin 2010, Doutrelant et al. 2016) (but see Avery et al. 2014). Across the southwest Pacific there are several polytypic radiations where sexual dichromatism and elaborate plumage coloration have apparently been differentially lost and gained on multiple isolated islands – e.g. *Turdus* spp (Peterson 2007), Solomon Islands *Monarcha* flycatchers (Uy et al. 2009, 2019), golden whistlers *Pachycephala* spp (Andersen et al. 2014b) and Pacific robin radiation (*Petroica multicolor* species complex) (Mayr 1963). These radiations offer a valuable natural experiment to explore the interplay between natural and sexual selection in driving reduced island dichromatism and sexual plumage elaboration (Peterson 1996, Grant 2001, Bennett and Owens 2002, Badyaev and Hill 2003).

Several non-mutually exclusive hypotheses are proposed to explain reduced island dichromatism. 1) Natural selection in response to increased predation risk and/or different environmental conditions on islands drives the loss of colorful plumage in both sexes in preference for more cryptic plumage (Soler and Moreno 2012, Shultz and Burns 2013). 2) Reduced sexual selection on islands compared to mainland causes the gradual loss of dichromatism – e.g. because of A) the loss of migratory drive and establishment of longer pair-bonds in sedentary island populations and/or B) increased extinction risk and small effective population sizes of populations with costly elaborate plumage (owing to only a small handful of males siring offspring) (McLain et al. 1995, Omland 1997, McLain et al. 1999, Griffith 2000, Badyaev and Hill 2003). 3) Founder effects result in random changes in sexual plumage color depending on the genes of the founders (Omland 1997). 4) Genetic drift results in more losses of costly elaborate plumage than gains (Badyaev and Hill 2003). At its extreme, different selective pressures on isolated islands could result in the gradual appearance of the geographic mosaics in sexual dichromatism in Pacific and other multi-island radiations even if all islands were initially founded from a dichromatic ancestor.

Here, we use multilocus DNA and plumage spectrophotometry to test these hypotheses in the evolutionary origins of the complex geographic mosaic of sexual plumage coloration in the Pacific robin

radiation (*Petroica multicolor* species complex). Pacific robins are a textbook example of the phenomenon of repeated gains and losses of sexual dichromatism in island taxa (Mayr 1963, Peterson 1996, Omland 1997, Futuyma 1997, Grant 2001). None of the other sexually dichromatic avian radiations (e.g. *Turdus* spp., Solomon Islands flycatchers, golden whistlers) approach the striking geographic mosaic of sexual plumage coloration of Pacific robins (Fig. 1). Sixteen distinct taxa are recognized in the Pacific robin species complex and recent molecular studies have identified three, or possibly four, distinct species (Mayr 1934, Mayr et al. 1937, Kearns et al. 2015, 2016) – Norfolk robin *P. multicolor* endemic to Norfolk Island and red-capped robin *P. goodenovii* endemic to mainland Australia are monotypic, while the Pacific robin *P. pusilla* has fourteen subspecies described from the Solomon Islands (and Bougainville Island), Vanuatu, Fiji and Samoa (Fig. 1).

Each distinct species/subspecies in the Pacific robin species complex varies in their degree of sexual dichromatism from taxa with marked differences between the sexes (marked sexual dichromatism) to taxa where both sexes are near monochromatic having either both sexes with 'feminized' dull brown plumage or both sexes with 'masculinized' elaborate black and red plumage (Fig. 1). Vanuatu has taxa with all three modes of sexual coloration, while Norfolk Island, Fiji and Samoa each have a single mode of sexual coloration (Fig. 1). Two modes of sexual coloration are present in the Solomon Islands, however, elaborate monochromatic plumage differs from that seen in Samoa and Vanuatu. Both *P. p. polymorpha* and *P. p. dennisi* have elaborate 'masculinized' females but with brown or russet heads rather than black heads (Fig. 1). Furthermore, *P. p. polymorpha* has two male color morphs – one with typical elaborate male plumage and one with a more melanized version of *P. p. polymorpha* females (Fig. 1) – it is unclear whether these two male morphs reflect birds of different ages (however, all are confirmed adults based on gonads) or if it is heritable and perhaps linked to different reproductive strategies (Mayr 1934).

Despite having influenced Ernst Mayr's theories on allopatric speciation and plumage evolution (ultimately leading to the development of the 'Biological Species Concept', Mayr 1942, 1963, Diamond 1970, Diamond and Mayr 1976), little is known of the evolutionary origins of variation in sexual dichromatism in Pacific robins owing to a lack of well-resolved taxon boundaries and phylogenetic relationships. Recent molecular studies have established that despite long being treated conspecific with the mainland Australian scarlet robin *P. boodang* (Mayr 1934, Schodde and Mason 1999), Pacific robins in the southwest Pacific instead form a species complex with mainland Australian red-capped robins *P. goodenovii*). Furthermore, the

taxon on Norfolk Island (Norfolk robin *P. multicolor*) represents a species distinct from the rest of the southwest Pacific taxa ("Pacific robin" *P. pusilla* with fourteen subspecies) (Kearns et al. 2016, 2019a). MtDNA and nuclear loci appear to further divide *P. pusilla* into two distinct lineages that might represent distinct species—one from the Solomon Islands and Bougainville Island (SOL), and the other from Vanuatu, Fiji and Samoa (VFS) (Kearns et al. 2016, 2019b, a). If species status is warranted, *Petroica polymorpha* Mayr, 1934 has precedence for the SOL lineage, and *Petroica pusilla* Peale, 1848 has precedence for the VFS lineage (see Kearns et al. 2016 for further justification).

Here, we examine variation in nuclear and mtDNA loci, morphometrics and plumage color across an extensive series of modern and historical museum specimens of Pacific robins – many of which were examined by Ernst Mayr in his seminal studies on this radiation (Mayr 1934, Mayr et al. 1937). We first use these data to test the species distinctiveness of the Solomon Islands (SOL) and Vanuatu/Fiji/Samoa (VFS) lineages using multilocus coalescent methods capable of modeling their history of divergence and gene flow. Second, we investigate the relationships between and quantify the distinctiveness of the fourteen named subspecies in the SOL and VFS lineages. Having established taxon boundaries and relationships, we then use these data to test the evolutionary origins of the complex geographic mosaic of sexual plumage coloration in robins across the Pacific. We focus on two key questions:

1) Was the ancestor of the entire Pacific robin radiation sexually dichromatic? If so, we expect to find a sexually dichromatic ancestor predicted for the node connecting all members of the Pacific robin species complex – i.e. connecting *P. goodenovii*, *P. multicolor*, and the SOL and VFS lineages currently ascribed to *P. pusilla*.

2) Did losses of sexual dichromatism occur multiple times in the SOL and VFS lineages? If so, we expect to find a) reconstruction of a sexually dichromatic ancestor for the SOL and VFS lineages, b) plumage differences between monochromatic taxa between the SOL and VFS lineages, c) differences in the degree of dichromatism/monochromatism between sexes in each monochromatic taxon and d) a lack of monophyly of populations with the same mode of sexual coloration. Alternatively, if island populations with the same mode of sexual coloration are each other's closest relatives this would suggest that these islands were founded by the same ancestor.

Material and methods

Genetic variation

Sampling approach and sequencing

Here we use the mtDNA *ND2* dataset previously published in Kearns et al. (2015, 2016) to examine range-wide patterns of phylogeographic structuring and genetic distinctiveness of all subspecies of Pacific robins. Previously, 1) Kearns et al. (2015) used these *ND2* data to test differentiation across robin subspecies in Vanuatu, which resulted in the description of a new subspecies (*Petroica pusilla tannensis* Kearns and Omland 2015), while Kearns et al. (2016) showed that the endangered Norfolk robins form a species complex with red-capped robins and SOL and VFS lineages of Pacific robins. Critically, Kearns et al. (2016) did not examine the phylogeographic structuring within the SOL and VFS lineages, which is the focus of our study. Here, we examine *ND2* sequences from 36 Pacific Robins (14 contemporary frozen tissue samples, 22 historical museum toepad samples), 5 Norfolk robins (all historical samples), and 4 red-capped robins (all contemporary samples) (GenBank accessions: KP203816-KP203833; MG676914 - MG676937). This included *ND2* sequences for all described subspecies of Pacific robins – Solomon Islands (n = 10): *polymorpha* n = 3, *kulambangrae* n = 3, *dennisi* n = 2, *septentrionalis* n = 2; Vanuatu (n = 17): *soror* n = 3, *ambrynensis* n = 3, *feminina* n = 3, *cognata* n = 3, *similis* n = 3, *tannensis* n = 2; Fiji (n = 7): *becki* n = 2, *kleinschmidti* n = 3, *taveunensis* n = 2; Samoa (n = 3): *pusilla* n = 3) (Supplementary Material Appendix 1, Table A1). Most of the historical specimens were collected between 1912 and 1953 as part of the Whitney South Seas Expedition and were examined in previous plumage-based studies (Mayr 1934, LeCroy 2008).

In addition to mtDNA, we examine five autosomal introns (GAPDH, CLTC, PCBD, CSDE, CLOCK) and two Z-linked sex chromosome introns (BRM, ACO1). Trials showed that the DNA from the historical specimens was too degraded to sequence nuclear introns. Our nuclear intron dataset therefore relies exclusively on the few contemporary tissues that have been collected for this radiation – such sparse sampling is typical for Pacific species with widespread, remote and fragmented distributions. We sample nuclear introns from 9 red-capped robins from Australia and 14 Pacific robins of which seven were from the SOL lineage (*polymorpha* n = 3, *kulambangrae* n = 4) and seven were from the VFS lineage (Vanuatu *ambrynensis* n = 3; Fiji *kleinschmidti* n = 3, *taveunensis* n=1) (Supplementary Material Appendix 1, Table A1). No fresh tissues were available from Samoa or Norfolk Island. Sequences for CLOCK and ACO1 were previously

published in (Kearns et al. 2016) (GenBank accessions: KT372722–KT372779). New sequences for the other five introns were produced for this study using the protocol described in Kearns et al. (2016) and with primers and annealing temperatures following previous studies (Borge et al. 2005, Kimball et al. 2009). All introns sequenced for this study were deposited in GenBank (accessions: BRM: MK121750-MK121772; GAPDH: MK127556-MK127575; PCBD: MK127576-MK127598; CSDE: MK248639-MK248660; CLTC: MK248661-MK248678).

Unrooted allele networks

Nuclear introns were phased using PHASE v2.1 (Stephens and Donnelly 2003) under the following settings—5 independent runs, repeating the final run 10 times, 70% probability threshold with uncertain heterozygous sites coded with IUPAC ambiguity codes. Sequences with length polymorphisms were resolved using the 'subtraction method' (Dolman and Moritz 2006). Several sequences in GAPDH, CLTC and CSDE had length polymorphisms that were too complex to resolve, and thus these sequences were omitted from further analyses. TOPALi v2.5 (Milne et al. 2004) was used to test for signals of recombination in the nuclear introns using the difference of sums-of-squares (DSS) method (sliding window: 100 bp; step size: 10 bp). No significant evidence of recombination was found in the seven introns. PopART (Leigh and Bryant 2015) was used to estimate unrooted allele networks for mtDNA and nuclear introns using an unrooted TCS haplotype network (Clement et al. 2000) calculated using default settings. DnaSP 5.10 (Rozas et al. 2003) was used to measure the net divergence in mtDNA *ND2* between taxa, islands and archipelagos using the *Dxy* statistic.

Multilocus tests of taxon boundaries and gene flow

Seven nuclear introns sampled from nine red-capped robins, seven SOL lineage (*polymorpha* n = 3, *kulambangrae* n = 4) and seven VFS lineage (Vanuatu *ambrynensis* n = 3; Fiji *kleinschmidti* n = 3, *taveunensis* n = 1) were used to test for taxon boundaries and introgression using STRUCTURE, IMa2 and species tree analyses. By using subspecies as the a priori taxonomic unit for species tree analyses in this study we are able to test the monophyly of each Pacific Robin lineage, which is important given that mtDNA phylogenies failed to offer strong support for the reciprocal monophyly of all the populations currently

circumscribed as *P. pusilla* (maximum likelihood trees place Norfolk and red-capped robins sister to the SOL lineage, while Bayesian trees only offered weak support for a sister relationship between the SOL and VFS lineages (posterior probability = 0.68); Kearns et al. 2016, 2019b, a). This approach differs from species tree analyses performed for Kearns et al. (2019b) across all of *Petroica* where SOL and VFS lineages were used as the a priori taxonomic units, which effectively enforced the monophyly of samples designated to each lineage (Kearns et al. 2019b). Critically, we were unable to sample nuclear introns from specimens from Samoa and Norfolk Island owing to the lack of fresh tissues and the degraded quality of the DNA obtained from museum skins. Thus, our nuclear analyses are restricted to testing the monophyly and relationships of samples from Vanuatu and Fiji rather than the entire VFS lineage.

STRUCTURE (Pritchard et al. 2000) was used to test population structuring in nuclear loci without needing to designate a priori taxon boundaries. PGDSpider v2.1.0.0 (Lischer and Excoffier 2011) was used to create the output files for STRUCTURE analyses using unique haplotypes from the seven nuclear introns. STRUCTURE analyses were performed with and without red-capped robins and under two alternative models – admixture with correlated allele frequencies, and no admixture with independent allele frequencies. Ten runs of one million generations with 500 000 generations discarded as burnin were performed for K = 1–4 for each model. CLUMPAK (Kopelman et al. 2015) was then used to test for the best value of K using both DeltaK and Ln(P)K metrics and to combine results for each K across independent runs using the CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2003) algorithms.

Species trees were estimated from the seven nuclear loci in *BEAST (Heled and Drummond 2010) implemented in BEAST v2.4.8 (Bouckaert et al. 2014) using a Yule speciation prior, a strict clock on all introns with an exponential prior for clock.rate, a lognormal prior on birthrate (M = 4.0, S = 1.25) and population mean (M = 5.0, S = 1.2), and subspecies as the a priori taxonomic unit. All introns used a HKY+I+G substitution model with empirical base frequencies and estimated values for kappa, gamma, shape and proportion of invariants. Two independent runs of 1×10^8 generations were performed with samples taken every 5000 generations. After omitting a burnin of 1×10^7 generations we used TRACER v1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) to assess whether the two independent runs had converged, reached stationarity and that ESSs were all above 100. Finally, we used LOGCOMBINER to combine the two

independent runs and then we estimated a maximum-clade-credibility tree (MCC) with mean heights using TREEANNOTATOR.

Demographic parameters for the SOL and VFS lineages (gene flow (2*NM*), divergence time (*t*) and population sizes (*Ne*)) were estimated from the seven nuclear introns using the 'Isolation-with-Migration-analytic model for more than two populations' (IMa2) (Hey 2009). Following initial test runs to optimize convergence, mixing, parameter priors, burnin and run length, three final replicate IMa2 runs were performed using 80 independent Markov-coupled chains with a geometric heating scheme (g1 = 0.96, g2 = 0.5), burnin of 100 000 steps and selecting an 'infinite' run duration recording output every 30 min. Each nuclear intron used a HKY model and mutation rate of 1.35 × 10^9 estimated for nuclear introns (Ellegren 2007). We assumed a generation time of one year in order to convert parameters to demographic units. Trial runs showed that we were unable to obtain closed posterior densities for ancestral population size (*q2*) and divergence time (*t*) with our data – *q2* maintained a flat curve indefinitely at a low posterior density, while *t* showed a distinct peak in posterior densities and then plateaued indefinitely without returning to zero. Accordingly, we opted to constrain the prior of *t* and *q2* to biologically meaningful values (following the recommendations of Won and Hey 2005). Final runs used the following parameter priors—population size (*q*) = 5, migration rate (*m*) = 10, and divergence time (*t*) = 2. The first final run was monitored and then stopped when adequate convergence and chain mixing had been reached (at 3 010 008 steps) as determined by a lack of trends in the L[P] and t parameter plots and ESS values above 10 000. The final two replicate runs were initiated with different starting seeds, used the same settings above and were run for 3 010 008 steps. Finally, we checked for consistency between the three replicate runs (following Hey 2005).

Phenotypic variation

Phenotypic variation was assessed for 186 Pacific Robins (116 males, 70 females) representing all fourteen described subspecies and 6 additional island populations in subspecies that occur on multiple islands. Of these, 49 (24 males, 25 females) were from the SOL lineage and 142 (93 males, 49 females) were from the VFS lineage (Vanuatu: 79, Fiji: 32, Samoa: 31). Kearns et al. (2016) previously examined differences between Norfolk robins, red-capped robins and Pacific robins, however, differences between and within Pacific robin lineages were not examined. Specimens were measured at the American Museum of Natural

History in New York, Delaware Museum of Natural History in Wilmington, Florida Museum of Natural History in Gainesville, and Academy of Natural Sciences at Drexel University in Philadelphia (Supplementary Material Appendix 1, Table A2). Most of the specimens examined were collected between 1912 and 1953 as part of the Whitney South Seas Expedition and were crucial to the seminal plumage-based taxonomic revision of Mayr (1934). Owing to the condition of some specimens, not all measurements were taken for all specimens (Supplementary Material Appendix 1, Table A2). Sufficient samples for quantitative tests were available for males and females of all subspecies except *P. p. taveunensis* of Fiji (Supplementary Material Appendix 1, Table A2).

We measured three morphometric variables (bill length, tail length, wing length) and reflectance of three plumage patches (crown, back, throat) using an Ocean Optics USB2000 reflectance spectrophotometer with a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, FA, USA). Three readings were taken for each plumage patch for each individual and then mean spectral reflectance curves (320–700 nm) calculated from these data were used to calculate tristimulus color variables for each (brightness 'B1', saturation 'S5a' and hue 'H4a') (Hill and McGraw 2006) using CLR v1.05 (Montgomerie 2008) (for details of protocols see Kearns et al. 2015, 2016). We tested for significance in variance using multivariate analysis of variance (MANOVA with Pillai test for significance) between 1) archipelagos in morphometric variables and 2) subspecies within each mode of sexual plumage coloration in plumage color variables – i.e. comparing all subspecies with marked dichromatism (elaborate males, dull females; 6 subspecies from Solomon Islands, Fiji and Vanuatu) versus all subspecies with dull monochromatism (dull 'feminized' males and females; 3 subspecies from Vanuatu) versus all subspecies with elaborate monochromatism (elaborate 'masculinized' males and females; 5 subspecies from Solomon Islands, Vanuatu and Samoa) (Fig. 1). We then identified the most discriminant variable between groups using linear discriminant analysis (LDA). To explore differences in the level of sexual dichromatism across subspecies with different plumage modes, we calculated standardized mean differences using Cohen's D and we tested for significant differences between the sexes using MANOVA. The two male plumage morphs of subspecies *P. p. polymorpha* were treated separately in all plumage analyses. We did not statistically test the distinctiveness of plumage color between each named subspecies owing to small sample sizes (Supplementary Material Appendix 1, Table A2). However, Tukey-HSD was used to further explore ANOVA results among subspecies within each island group with

significance levels adjusted using Bonferroni correction in order to control for multiple comparisons across the non-independent tristimulus color variables (hue, saturation, brightness) ateach plumage patch ($p = 0.05/3 = 0.0167$) (analyses performed in JMP v10). All other statistics and plots were performed in R v3.5.1 using the MASS, ggplot2, dplyr, ggord and lsr packages.

Reconstruction of ancestral sexual plumage coloration

Ancestral state reconstructions of plumage were performed in Mesquite v3.51 (Maddison and Maddison 2003) using both parsimony and likelihood (Markov k-state one parameter model; Lewis 2001), which both use an equal rate of character change. We considered two phylogenies of *Petroica*. One phylogeny was focused at the species-level, which is primarily based on a species tree analysis of two mtDNA loci and five nuclear introns (from Kearns et al. 2019b), but with the position of missing *P. multicolor*, *P. archboldi* and *P. bivittata* inferred solely from their position in phylogenies based on mtDNA *ND2* (Kearns et al. 2019a) (for further discussion see Kearns et al. 2019b). The second phylogeny focused at the subspecies-level, added subspecies to the phylogeny based on their relationships inferred from mtDNA in this (Fig. 2) and other studies (Miller and Lambert 2006, Kearns et al. 2019a, 2019b). We performed two reconstructions of ancestral plumage examining either: 1) the three modes of sexual coloration or 2) the presence or absence of elaborate plumage in at least one sex (i.e., irrespective of dichromatism level).

For ancestral state reconstructions, we defined elaborate sexually monochromatic plumage as both sexes having heavily melanized feathers on the dorsal surfaces (males: black; females: black or dark grey), and dull sexually monochromatic plumage as both sexes having lightly melanized feathers on the dorsal surfaces (both sexes: brown or light grey). As such, we classified New Zealand's *P. australis* and *P. longipes,* and New Guinea's *P. archboldi* as dull sexually monochromatic, while New Guinea's *P. bivittata* was classified as elaborate sexually monochromatic. Note that many species/subspecies classified as sexually elaborate and dull monochromatic show some minor differences between the sexes, however, these differences do not approach those of the marked sexually dichromatic mode.

RESULTS

Species distinctiveness of SOL and VFS lineages

Differentiation at mtDNA and nuclear loci

The VFS and SOL lineages were 3.85% divergent in mtDNA *ND2* (Fig. 2, Table 1). We did not have complete taxon sampling for the nuclear dataset, and thus the VFS lineage is represented by *ambrynensis* from Vanuatu and *kleinschmidti* and *taveunensis* from Fiji (henceforward the VF(S) lineage), while the SOL lineage is represented by *kulambangrae* and *polymorpha*. Three nuclear introns (CLOCK, PCBD, CSDE) had no shared haplotypes between the VF(S) and SOL lineages (Supplementary Material Appendix 1, Fig. 1). The other four nuclear introns had shared haplotypes between the two lineages (Supplementary Material Appendix 1, Fig. 1), however, all shared alleles were internal in the network suggesting sharing is likely caused by the retention of ancestral alleles rather than gene flow (Omland et al. 2006). Red-capped robins were either several mutational steps divergent from all Pacific robins in nuclear introns or only shared a single allele with VF(S)/SOL that was internal in the network (Supplementary Material Appendix 1, Fig. 1).

Multilocus tests of lineage/species boundaries

Models using admixture and correlated frequencies selected two populations (K = 2) as the best fit K using the DeltaK method, and four populations (K = 4) as the best fit K using the Ln(P)K method (Fig. 3). For admixture models including Red-capped Robins, K = 2 differentiated Pacific robins and red-capped robins, but placed subspecies *polymorpha* from the Solomon Islands intermediate to these two clusters (Fig. 3). The SOL and VF(S) lineages formed distinct clusters at K = 3, and both *polymorpha* and *kulambangrae* from the Solomon Islands formed distinct clusters at K = 4 (Fig. 3). Admixture models excluding red-capped robins (not shown) inferred similar structuring to those including red-capped robins – differentiating Solomon Islands *kulambangrae* vs the rest of the Pacific robins at K = 2, *polymorpha* vs *kulambangrae* vs Vanuatu/Fiji at K = 3 and *polymorpha* vs *kulambangrae* vs Vanuatu vs Fiji (with some ambiguity) at K = 4. Models using no admixture and independent allele frequencies (not shown) both selected two populations (K = 2) as the best-fit K – differentiating either red-capped robins from all Pacific robins when red-capped robins were included and differentiating all Solomon Islands robins from robins in Vanuatua and Fiji when red-capped robins were excluded.

Multilocus coalescent species trees estimated from all seven nuclear introns supported the reciprocal monophyly of all robins in the southwest Pacific to the exclusion of red-capped robins (Fig. 3). The monophyly of the SOL lineage (represented by *polymorpha* and *kulambangrae*) was strongly supported (posterior probability = 0.95), whereas there was weaker support for the monophyly of the VF(S) lineage (represented by *ambrynensis* from Vanuatu, and *kleinschmidti* and *taveunensis* from Fiji) (posterior probability = 0.69) (Fig. 3).

IMa2 estimated that the SOL and VF(S) lineages diverged around 400 000 years ago (High point = 377 915) (Fig. 3). Broad 95% highest posterior density (HPD) credibility intervals were estimated (235 022 – 3 758 467 ya) likely stemming from issues obtaining closed posterior densities for divergence time (*t*). Notably, although the posterior densities for *t* failed to return to zero, posterior densities were substantially lower than the high point after 1 million years (Fig. 3). Thus, the divergence of the SOL and VF(S) lineages most likely occurred 235 022 – 1 000 000 years ago. Population migration rate (2*NM*) was estimated to be very low between the SOL and VF(S) lineages (Fig. 3). Posterior densities included zero and an assumption of zero gene flow ($m = 0$) could not be rejected by likelihood ratio (LLR) tests performed within IMa2. Effective population size (*Ne*) estimates were smaller for SOL (mean = 127 273; 95% HPD credibility intervals = 31 728 – 243 247) than for VF(S) (mean = 449 631; 95% HPD credibility intervals = 165 690 – 790 848). We were unable to get an accurate estimate of ancestral population size (mean = 863 163; 95% HPD credibility intervals = 0 – 2 071 716), however, the high point of posterior densities appeared within a similar range as the VF(S) lineage. Our issues achieving closed posterior densities for several parameters no doubt stem from our small sample sizes (both the small number of nuclear loci used and our limited taxon and individual sampling). Given these issues, our results therefore need to be interpreted as broad preliminary estimates of the historical dynamics between these two lineages.

Phenotypic variation

Morphometric variables were significantly differentiated across the archipelagos (Table 2), however, LDA did not clearly divide the SOL and VFS lineages (Supplementary Material Appendix 1, Fig. 5A). Instead, morphometric variation divided smaller-bodied birds from the Solomon Islands, Fiji and Samoa from larger-bodied birds from Vanuatu (Supplementary Material Appendix 1, Fig. 2). Males from Vanuatu had

significantly longer wing lengths compared to other archipelagos (pairwise Tukey HSD: all $p < 0.0001$), however, after Bonferroni correction, only females from Vanuatu were statistically distinguishable from those from the Solomon Islands ($p < 0.02$). Birds from Fiji, Samoa and the Solomon Islands were not statistically distinguishable in morphometrics after Bonferroni correction. We found significant differences between subspecies from SOL and VFS within each mode of sexual plumage coloration (Table 3), but owing to the complexity of plumage variation in this species complex it is challenging to associate these as differences between SOL and VFS lineages per se (details below) (Fig. 4, Supplementary Material Appendix 1, Fig. 3–5).

Subspecies distinctiveness within the SOL lineage

Subspecies in the SOL lineage were deeply divergent in both mtDNA (*Dxy* = 1.8–2.5%; Table 1, Fig. 2) and nuclear introns (Supplementary Material Appendix 1, Fig. 1). The deepest mtDNA divergences were between southernmost *polymorpha* versus each of the other subspecies in the Solomon Islands – *dennisi* (2.5%), *kulambangrae* (2.2%), *septentrionalis* (2.4%) (Fig. 2, Table 1). MtDNA divergences for *kulambangrae*, *septentrionalis*, and *dennisi* ranged between 1.8–1.9% (Table 1). Despite their distinctiveness there was poor topological support for mtDNA relationships among the Solomon Islands subspecies (Bayesian PP < 0.76, ML bootstrap < 68) (Kearns et al. 2016). Nuclear introns also showed structuring between *kulambangrae* and *polymorpha* (Supplementary Material Appendix 1, Fig. 1) – at least one of these subspecies had unique alleles at each locus, and there were no shared haplotypes between *kulambangrae* and *polymorpha* or any other subspecies at both CSDE and CLOCK. STRUCTURE analyses showed population structuring between *kulambangrae* and *polymorpha* (Fig. 3). Subspecies in the Solomon Islands also showed statistically significant differences in wing length and tail length, but not bill length (Wing: ANOVA – female: $F = 11.25$, $p = 0.0003$, df = 3,17; male: $F = 12.92$, $p < 0.0001$, df = 3, 19; Tail: ANOVA – female: $F = 7.79$, p 0.0017, df = 3,17; male: $F = 14.50$, $p < 0.0001$, df = 3,19; Bill: ANOVA – female: $F = 0.33$, $p = 0.80$, df = 3,16; male: $F = 0.50$, $p = 0.69$, df = 3,17) (Supplementary Material Appendix 1, Fig. 2). SOL subspecies overlapped in LDA analyses of plumage with the exception of russet-headed *P. p. polymorpha* males, and also females from *kulambangrae* and *septentrionalis*, however, these were based on small sample sizes and thus have limited power (Supplementary Material Appendix 1, Fig. 5).

Subspecies distinctiveness within the VFS lineage

Subspecies in the VFS lineage showed shallower divergences compared to those in the SOL lineage (mtDNA *Dxy* = 0.16–0.96%). Vanuatu, Fiji and Samoa archipelagos were not reciprocally monophyletic in mtDNA (Fig. 2, Kearns et al. 2016) nor were representatives from Vanuatu and Fiji inferred to form distinct clusters in STRUCTURE analyses of nuclear introns (Fig. 3). We found that all Vanuatu subspecies except *soror* radiated from a mtDNA haplotype sampled from a single *feminina* individual, while sequences from Samoa and Fiji are at least 3 substitutions different from this central Vanuatu haplotype (Fig. 2). MtDNA and nuclear DNA did however show some evidence of early stages of differentiation since there were no shared mtDNA haplotypes (Fig. 2) and many unique alleles in the other nuclear introns (Supplementary Material Appendix 1, Fig. 1).

In Vanuatu, subspecies showed mtDNA divergence (Fig. 2, Table 1) and previous detailed analyses by Kearns et al. (2015) found that subspecies also differed across several morphometric and plumage color traits – even among some subspecies with the same mode of sexual plumage coloration (e.g. elaborate *similis* and *tannensis*). In Samoa, samples from Upolu and Savai'i islands had unique *ND2* haplotypes that were 0.21% divergent from each other (Fig. 2, Table 1), but did not differ in plumage, wing, tail or bill length after Bonferroni correction (Supplementary Material Appendix 1, Fig. 2–5). In Fiji, mtDNA divided subspecies *kleinschmidti* into two paraphyletic lineages that were more divergent (0.87%) than most pairwise comparisons between the archipelagos of Vanuatu, Samoa and Fiji (*ND2 Dxy* = 0.66−0.75%, Table 1). Subspecies within each paraphyletic lineage had unique mtDNA haplotypes – *kleinschmidti* from Vanua Levu and *taveunensis* were 0.16% divergent, *kleinschmidti* from Viti Levu and *becki* were 0.32% divergent. Notably, mtDNA paraphyly was apparent in samples originating from both contemporary tissues and historical samples (Supplementary Material Appendix 1, Table A2). Though sample size is small, nuclear introns do not differentiate *kleinschmidti* from Vanua Levu (n = 1) and Viti Levu (n = 2). Tukey-HSD showed no differences in morphometric or plumage measurements across subspecies and isolated islands in Fiji, however, these tests had limited power owing to small sample sizes (Supplementary Material Appendix 1, Fig. 2–5).

Evolution of plumage color

Was the ancestor of the entire Pacific robin radiation sexually dichromatic?

Likelihood and parsimony reconstructions on the species-level phylogeny support an ancestor with marked sexual dichromatism for the ancestor of the Pacific robin species complex and for the ancestor that diverged into the SOL and VFS lineages (Fig. 5A, Supplementary Material Appendix 1, Fig. 6). While reconstruction of the three modes of sexual plumage coloration was equivocal under the likelihood model for the subspecies-level phylogeny (Fig. 5B), parsimony reconstructions gave equal probability to either a marked dichromatic or elaborate monochromatic ancestor (Supplementary Material Appendix 1, Fig. 7A). In contrast, all reconstructions of the presence or absence of elaborate plumage supported an ancestor with at least one sex with elaborate plumage for all of *Petroica*, for the ancestor of the entire Pacific robin radiation, for the ancestor that diverged into the SOL and VFS lineages, and for the ancestor of both SOL and VFS lineages (Fig. 5, Supplementary Material Appendix 1, Fig. 6–7).

Are there plumage differences between and within monochromatic taxa from the SOL and VFS lineages?

Within the elaborate monochromatic plumage mode, MANOVA based on all patches and color variables found significant differences between subspecies in females (MANOVA Pillai test $p < 3.894e-07$) and in both subsets of males either including or excluding the russet-headed male morph of *P. p. polymorpha* (black-headed only: $p < 0.000184$; all males: $p < 3.874e-09$) (Table 3, Fig 4; Supplementary Material Appendix 1, Fig. 3–4). Individual ANOVAs show that differences are not only present in the variable crown and throat plumage patches (Fig. 1), but also in the back plumage patch (Table 3, Fig 4, Supplementary Material Appendix 1, Fig. 3–4). LDA showed discrete differentiation of subspecies between, but not within, archipelagos among elaborate females involving especially crown and throat hue in one direction and back hue in another direction (Supplementary Material Appendix 1, Fig. 5D). Unsurprisingly, LDA strongly differentiated the russet-headed male morph of *P. p. polymorpha* from all black-headed elaborate males along the LD1 axis associated mostly with crown saturation and hue (Supplementary Material Appendix 1, Fig. 5D). Among the black-headed elaborate males, patterns were similar to elaborate females, but with much more overlap between subspecies (Supplementary Material Appendix 1, Fig. 5D).

Within the dull monochromatic plumage mode, MANOVA based on all patches and colour variables failed to find significant differences between subspecies in both males ($p < 0.15$) and females ($p < 0.053$) (Table 3, Fig 4, Supplementary Material Appendix 1, Fig. 3–4). LDA showed discrete differentiation with no overlap between subspecies in dull monochromatic females, but differentiation was less strong for dull monochromatic males where larger sample sizes were available (Supplementary Material Appendix 1, Fig. 5C).

For comparison to the monochromatic plumage modes, we also evaluated the degree of differentiation between subspecies within the marked sexual dichromatism plumage mode. MANOVA based on all patches and colour variables found significant differences between dichromatic subspecies in males ($p < 0.04$), but not in females ($p < 0.07$) (Table 3, Fig 4, Supplementary Material Appendix 1, Fig. 3–4). Conversely, LDA showed no discrete differentiation of subspecies among dichromatic males, whereas dichromatic females showed some differentiation albeit based on small sample sizes, which could enhance the perception of differentiation between groups (Supplementary Material Appendix 1, Fig. 5B).

Are there differences in the degree of dichromatism between sexes in each monochromatic taxon?

Tests of the level of sexual dichromatism in plumage revealed that even 'monochromatic' subspecies are in fact sexually dichromatic, though to a much lesser degree than marked sexually dichromatic taxa (Table 4, Supplementary Material Appendix 1, Table A3). Standardized mean differences between sexes were substantially larger in dichromatic subspecies than monochromatic subspecies, however, the sexes were significant differentiated in monochromatic subspecies across several variables (Table 4). In monochromatic subspecies, sexual dichromatism was most accentuated in crown and throat plumage patches compared to back plumage (Table 4, Fig. 4, Supplementary Material Appendix 1, Fig. 3–4). Even so, significant differences were found in back plumage in all elaborate subspecies except *P. p. polymorpha.* In contrast, the back plumage of dull monochromatic subspecies was not significantly different (Table 4).

DISCUSSION

Our goal was to resolve species boundaries and explore the evolutionary history of the Pacific robin radiation, and in particular test hypotheses about the origins of the complex geographic mosaic of sexual plumage

coloration in the Pacific robin radiation – a pattern that has intrigued evolutionary biologists and ornithologists for decades (Mayr 1934). Based on nuclear loci, mtDNA, quantitative measures of plumage coloration and reconstruction of ancestral plumage, we hypothesize that the Pacific robins were most likely founded from a sexually dichromatic ancestor and that the geographic mosaic of sexual plumage coloration has originated via repeated independent gains of elaborate plumage in females and losses of elaborate plumage in males. In addition, our molecular analyses offer clear support for the evolutionary distinctiveness of the SOL and VFS lineages, which are estimated to have diverged around 400,000 ya with no gene flow (Fig. 3C–D). We follow Kearns et al. (2016) in recommending their recognition as two distinct species – *Petroica polymorpha* Mayr, 1934 for the SOL lineage and *Petroica pusilla* Peale, 1848 for the VFS lineage (see further justification below).

Colonization history

Overall, it appears most likely that the SOL and VFS lineages diverged following a single radiation across the Pacific. However, there is some uncertainty stemming from conflicting support for relationships within the entire Pacific robin radiation when Norfolk robins are included in mtDNA analyses (Kearns et al. 2016, 2019a). Denser taxonomic sampling of nuclear loci including the Norfolk Robin and all subspecies within the SOL and VFS lineages will be required to ultimately test whether the VFS and SOL lineages differentiated following a single Pacific-wide radiation or originated from two separate dispersals from New Guinea or Australia. Irrespective of this, we find little molecular evidence for dispersal/geneflow among archipelagos and islands following the initial colonization of the southwest Pacific. Two possible exceptions are the divergent mtDNA lineages in Fiji (paraphyletic *kleinschmidti*) and Vanuatu (*soror* versus the five other Vanuatu subspecies), which suggest that some gene flow or secondary colonizations could have occurred in these archipelagos after the initial radiation. Alternatively, such patterns could result after a single founding wave of colonization if there was incomplete lineage sorting or differential fixation of divergent haplotypes owing to differences in genetic drift and founder effects on isolated islands.

The star-shaped polytomy in mtDNA in the VFS lineage is indicative of a recent radiation that could still retain unsorted ancestral polymorphisms (Fig. 2). Similar mtDNA polytomies have been found in other Pacific radiations and have been argued to reflect a process wherein colonization of archipelagos occurred

near simultaneously with little or no gene flow between islands following this initial wave of colonization (Grant 2001, Andersen et al. 2014b). In contrast, the subspecies within the SOL lineage are more differentiated and appear to have been isolated for a longer period of time than those in VFS. Makira Island *polymorpha* was most divergent (Fig. 2–3), which mirrors patterns in other Solomon Island radiations such as *Monarcha* flycatchers (Uy et al. 2009) and Golden Whistlers (Andersen et al. 2014b). This is consistent with models of sea-level change during the Pleistocene glacial cycles (18 ka – 2 mya), which predict that Makira Island remained isolated even during times of lower sea levels when the northern islands were connected and robins would have more easily exchanged genes (Hope 1996). Overall, however, it is striking how recently diverged the Pacific robin radiation is compared to the deeper divergences seen in other Pacific radiations – e.g. Pacific honeyeaters (Andersen et al. 2014a), kingfishers (Andersen et al. 2013) and golden whistlers (Andersen et al. 2014b) – although the radiation of Pacific reed warblers is likely on a similar recent time-scale (Cibois et al. 2011).

Evolution of plumage color

Was the ancestor of the entire Pacific robin radiation sexually dichromatic?

Ancestral state reconstructions of sexual plumage coloration in *Petroica* overwhelmingly support elaborate plumage (in either or both sexes) as an ancestral state throughout the *Petroica* phylogeny, including for the Pacific robin radiation (Fig. 5). This strongly suggests that elaborate plumage has been secondarily lost in entirely dull plumaged subspecies and species. Reconstructions of the three modes of sexual plumage color are less straightforward, however, on balance there is most support for a marked sexually dichromatic ancestor of both the entire Pacific robin species complex (i.e. including *P. goodenovii* and *P. multicolor*) as well as the ancestor of SOL and VFS (Supplementary Material Appendix 1, Fig. 6–7). A dichromatic ancestor is also highly likely since even monochromatic taxa have minor levels of sexual dichromatism – i.e. our spectrophotometry measures show that females are lighter than males in all elaborate and dull monochromatic taxa (Table 4, Fig. 4, Supplementary Material Appendix 1, Fig. 3–4) (Eaton 2005). Additionally, across all *Petroica*, species with marked sexually dichromatic plumage dominate the phylogeny (Australia: 5 species; New Zealand: 2 species; Pacific: 1 species) and three of six species with monochromatic plumage are polytypic and have both sexually dichromatic and monochromatic coloration (Boles 2007).

Did losses of sexual dichromatism occur multiple times in the SOL and VFS lineages?

Within Pacific robins, our data support multiple independent losses of sexual dichromatism in the SOL and VFS lineages and likely even multiple losses within archipelagos – all over a relatively recent time scale. Namely, we find a) support for a sexually dichromatic ancestor (as discussed above), b) plumage differences between monochromatic taxa between and within the SOL and VFS lineages (Table 3) (Furthermore, LDA of elaborate females discretely differentiated subspecies from different archipelagos; Supplementary Material Appendix 1, Fig. 5D), c) differences in the degree of dichromatism/monochromatism between sexes in each monochromatic taxon (Table 4) and d) a lack of monophyly of populations with the same mode of sexual coloration (Fig. 2–3).

Our finding of high variability of elaborate monochromatic plumage on different archipelagos (Fig. 4, Supplementary Material Appendix 1, Fig. 3–5, Table 4) is consistent with at least three independent origins of elaborate monochromatism – one each on Vanuatu, Samoa and Solomon Islands. However, it is unclear whether taxa within these archipelagos evolved elaborate monochromatism independently. For example, the distinctive feminized heads in elaborate monochromatic *dennisi* and *polymorpha* in the SOL lineage could have evolved independently owing to similar selective pressures or it could result from common ancestry given that relationships among subspecies in Solomon Islands were equivocal in our study and previous phylogenetic analyses (Kearns et al. 2016, 2019a, 2019b). A third alternative, which appears to be supported by ancestral state reconstructions (Fig. 5B) is that the ancestor of the SOL lineage had elaborate monochromatic plumage and that dichromatic *kulambangrae* and *septentrionalis* could have secondarily lost black backs in females. Similarly, ancestral state reconstructions do not clearly favor an ancestral mode of sexual coloration for the VFS lineage – two scenarios seem plausible: either 1) a marked dichromatic or elaborate monochromatic ancestor with at least two independent losses of elaborate plumage (*soror* versus *cognata/feminina*) or 2) a dull monochromatic ancestor with independent gains of elaborate plumage in *ambrynensis* (dichromatic) and *tannensis/similis* (elaborate monochromatic).

What has driven the evolution of plumage coloration in Pacific robins?

Island colonization has clearly played a role in shaping the evolution of plumage coloration in *Petroica* – all six monochromatic species within *Petroica* occur on islands (New Guinea: 2 species; New Zealand: 2 species; Pacific: 2 species) (Kearns et al. 2019a, 2019b). This pattern fits with global observations of the phenomena of reduced sexual dichromatism on islands (Omland 1997, Figuerola and Green 2000, Badyaev and Hill 2003, Roulin and Salamin 2010, Doutrelant et al. 2016), which is hypothesized to result from four non-mutually exclusive selective processes on islands: 1) increased natural selection, 2) reduced sexual selection, 3) founder effects and 4) random genetic drift (Omland 1997, McLain et al. 1999, Griffith 2000, Badyaev and Hill 2003, Soler and Moreno 2012, Shultz and Burns 2013). Overall, it appears unlikely that genetic drift has been the dominating driver of losses of sexual dichromatism in Pacific robins since genetic drift is predicted to drive more losses of costly elaborate plumage than gains and in Pacific robins more taxa have elaborate monochromatic plumage than dull monochromatic plumage (Badyaev and Hill 2003). Instead, it is more likely that a mixture of founder effects, natural selection and/or reduced sexual selection have led to the prevalence of Pacific robin taxa with elaborate monochromatic plumage since elaborate plumage is not negatively selected under these processes (Badyaev and Hill 2003). Relaxation or strengthening of different selection pressures (e.g. reduced predation on islands, and/or increased territorial competition that affects both females and males) on different islands could create a geographic mosaic in sexual dichromatism across the Pacific robin radiation by causing some island taxa to keep ancestral sexual dichromatism and others to lose elaborate male plumage or gain elaborate female plumage. Selection and genetic drift often act together (Bennett and Owens 2002), and as such differential losses or gains of sexual dichromatism in island taxa can be driven by purely random differences in the direction of selection and/or genetic drift (Peterson 1996, Omland 1997, Grant 2001, Bennett and Owens 2002, Badyaev and Hill 2003). This is because phenotypic and genetic differences can quickly fix in island taxa, which typically are isolated, have small population sizes, low genetic diversity and high rates of inbreeding (Grant 2001). That we also found plumage variation between subspecies with marked dichromatic plumage (Fig. 4, Supplementary Material Appendix 1, Fig. 3–5, Table 3) suggests that such random and diverse processes are influencing plumage evolution in the Pacific robin radiation (Peterson 1996, Omland 1997, Grant 2001, Bennett and Owens 2002, Badyaev and Hill 2003).

Little is known about the underlying genetic and mechanistic causes of changes in sexual dichromatism (Owens and Short 1995, Kimball and Ligon 1999, Badyaev and Hill 2003). However, it is

possible that even if a trait like elaborate plumage was lost in a distant ancestor, its genetic basis can be retained in the genome, potentially allowing it to be re-expressed in descendant taxa in a seemingly independent way (Raikow et al. 1979, Marshall et al. 1994, Wiens 2011). This is certainly a possibility for *Petroica* and the Pacific robins in particular, since all three modes of sexual coloration are scattered across the phylogeny and have seemingly evolved multiple times in parallel but slightly different ways. A genomic approach comparing multiple *Petroica* lineages with different modes of sexual plumage coloration would undoubtedly provide novel insights into our developing understanding of the genomic basis of plumage evolution and sexual dichromatism (Husby et al. 2012, Roulin and Ducrest 2013, Huang and Rabosky 2014, Tringali et al. 2015, Uy et al. 2016, Charmantier et al. 2017, Uy et al. 2019).

Taxonomic implications

Our genetic and phenotypic data are consistent with treating the SOL and VFS lineages as distinct species. Namely, SOL and VFS have 1) reciprocally monophyletic and deeply divergent mtDNA (Fig. 2, Table 1), 2) few shared nuclear alleles and two nuclear loci with fixed differences (Supplementary Material Appendix 1, Fig. 1), 3) support for two distinct lineages/clusters in STRUCTURE and species tree analyses (Fig. 3A–B), 4) IMa2 estimates of divergence with a lack of post-divergence gene flow (Fig. 3D), and 5) differences in elaborate monochromatic plumage (i.e. in SOL all elaborate subspecies have brown- or russet-headed females, while in VFS all elaborate subspecies have females with black heads) (Fig. 4, Supplementary Material Appendix 1, Fig. 3–5). Acknowledging our small sample sizes and incomplete taxon sampling in nuclear DNA, we still believe that each of these lines of evidence demonstrate the long-term, independent, evolutionary history and likely reproductive isolation of the SOL and VFS lineages and support their treatment as separate species consistent with (Gill 2014) and under the Generalized Lineage, Evolutionary and Phylogenetic species concepts. Following Kearns et al. (2016), we recommend the following English common names and species epithets: “Solomon robin” *Petroica polymorpha* Mayr, 1934 for the SOL lineage and “Mayr's robin” *Petroica pusilla* Peale, 1848 for the VFS lineage. Notably, a lack of other diagnosable differences in plumage and morphometric traits between the SOL and VFS lineages is not surprising given that these traits were the basis of the taxonomy that long united Norfolk robins and Pacific robins with scarlet robins (*P. boodang*) in a single polytypic and polyphyletic species (*P. multicolor* sensu Mayr 1934).

Molecular analyses have subsequently shown that Scarlet Robins belong to a lineage endemic to Australia, and which is deeply divergent from the Pacific robin radiation (Kearns et al. 2016). Convergence, reversal and stasis in plumage patterns are well documented for a wide range of avian taxa (reviewed by Omland and Lanyon 2000). Furthermore, similar findings of discordance between morphology and genetics have been seen at both species-level and higher taxonomic levels in the Pacific—e.g., Golden Whistlers (*Pachycephala* sp.) (Andersen et al. 2014b), Pacific Meliphagidae honeyeaters (Andersen et al. 2014a), and Solomon Islands flycatchers (*Monarcha* sp.) (Uy et al. 2019).

We find support for the distinctiveness of the thirteen subspecies that were described by Mayr (1934, Mayr et al. 1937), as well as *tannensis* that we recently described from Vanuatu in Kearns et al. (2015) – with a possible caveat regarding paraphyletic *kleinschmidti* from Fiji (below). Distinctiveness of subspecies based on plumage color was too difficult to disentangle from sexual plumage coloration mode (Fig. 4, Supplementary Material Appendix 1, Fig. 3–5), and our small sample sizes did not allow tests for statistical differences in plumage or morphometrics (Supplementary Material Appendix 1, Table A2 for sample sizes). However, all fourteen subspecies had distinct mtDNA haplotypes (Fig. 2; *ND2 Dxy* = 0.16–2.5) and there was evidence of nuclear differentiation in the five subspecies for which we had fresh tissues and nuclear sequences (Fig. 3, Supplementary Material Appendix 1, Fig. 1). Denser taxonomic sampling will be required to test whether populations on isolated islands in Fiji and Samoa represent distinct taxa that should be recognized at the subspecific level. The two paraphyletic mtDNA lineages found on Vanua Levu and Viti Levu islands could represent distinct subspecies with disparate evolutionary histories or these patterns could result from incomplete lineage sorting or gene flow following secondary colonization of Fiji from elsewhere in the Pacific (Fig. 2). Likewise, Samoan populations on Upolu and Savai'i islands could represent distinct subspecies since their mtDNA divergence (*Dxy* = 0.21%) was of a similar level as that observed between other subspecies in Vanuatu (*Dxy* = 0.18–0.64%) and Fiji (*Dxy* = 0.16–0.96%) (Fig. 2). Critically, the low level of divergence of the Samoan subspecies *P. p. pusilla* compared to other subspecies and species in the Pacific robin radiation is not consistent with recognizing this taxon as a distinct species as recently proposed based on their distinctive behavior and song (Pratt and Mittermeier 2016).

Conclusions

Modern molecular techniques now show that the radiations and archipelagic colonizations of the so-called 'great-speciators' of the Pacific have occurred on multiple temporal and spatial scales (Filardi and Moyle 2005, Smith and Filardi 2007, Kirchman and Franklin 2007, Jones and Kennedy 2008, Jønsson et al. 2008, Uy et al. 2009, Moyle et al. 2009, Clegg and Phillimore 2010, Andersen et al. 2013a, 2013b, 2015a, 2015b). Differences in how natural and sexual selection and genetic drift interplay in each species and on each isolated island have led to discordant and concordant signatures of phenotypic and genetic divergence. Given that one of the most pronounced signals from the Pacific avifauna is one of radiation-specific idiosyncrasy, it is clear that more robust phylogenetic frameworks need to be developed. Without such new datasets we will be unable to determine the extent to which speciation in these insular radiations has occurred via concerted responses to local selective pressures or to random drift and chance. This should be a priority, not just because species in the Pacific are increasingly threatened by habitat loss, climate change and predation (Kingsford et al. 2009), but also because these radiations hold historical importance to the fields of ornithology and speciation in general (Mayr 1934, 1942). Our findings here add to our understanding of the evolution of sexual plumage coloration in island versus mainland taxa and help to resolve the taxonomy of the iconic Pacific robin radiation, which ultimately will help to conserve threatened robin populations across the Pacific (Garnett et al. 2010, BirdLife International 2012).

Data availability statement

All new sequence data is deposited in GenBank under the following accession numbers (MK121750-MK121772; MK127556-MK127598; MK248639- MK248678). Raw phenotypic measurements are available upon request.

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Supplementary material (Appendix JAV-02404 at <www.avianbiology.org/appendix/jav-02404>). Appendix 1.

Figure Legends

Figure 1. Geographic mosaic of sexual dichromatism across the Pacific robin radiation. Insets illustrate the three different modes of sexual coloration across the Pacific robin radiation – i.e. Norfolk robin *P. multicolor* (NI: pink), red-capped robin *P. goodenovii* (RC: black and white) and Pacific robin *P. pusilla*, which is divided into recognized subspecies in the Solomon Islands (SOL: blue), Vanuatu (V: orange), Fiji (F: purple) and Samoa (S: green) archipelagos. Sex symbols indicate whether the species/subspecies has marked sexual dichromatism (elaborate males, dull females), dull monochromatism (dull 'feminized' males and females) or elaborate monochromatism (elaborate 'masculinized' males and females) (black = elaborate; white = dull). Cartoon birds illustrate the male plumage form followed by the female plumage form for all subspecies except for *polymorpha* where the two types of male plumage are illustrated followed by female plumage.

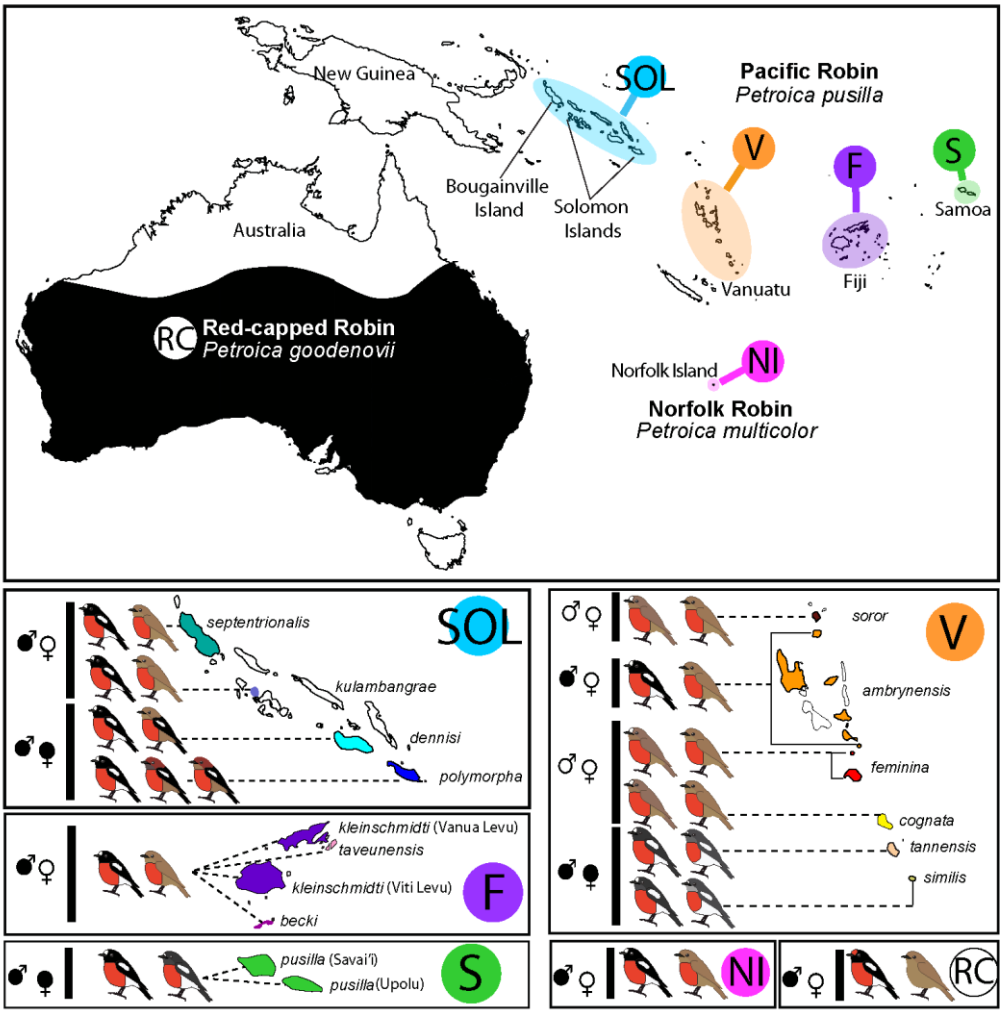

Figure 1.

Figure 2. Phylogeographic structuring of mtDNA ND2 across the Pacific robin species group. Haplotypes are scaled by sample size and colored and labeled by subspecies. Labels use the first letter of the archipelago and subspecies, except for *soror* (VSo) and *similis* (VSi), and subspecies from the SOL lineage (So) and Samoa (Sa). See inset for key. Subspecies with multiple islands sampled are indicated. Predicted unsampled haplotypes are represented by black circles and the number of mutations between haplotypes are indicated by hatched lines.

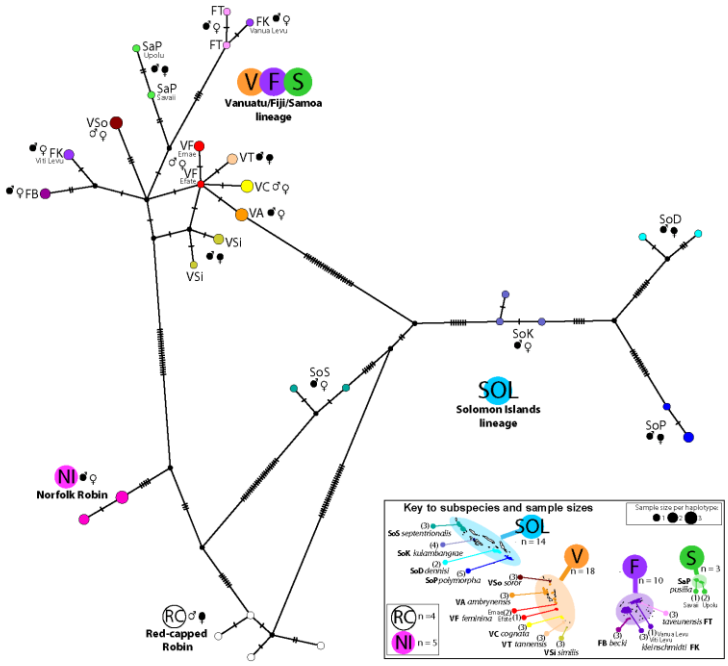

Figure 2.

Figure 3. Nuclear multilocus tests of taxon boundaries. (A) STRUCTURE plots showing assignment of each individual (represented by a vertical bar) to a population at each value of K. Delta K predicted that the best value of K was two populations, whereas Ln(P)K selected four populations as the best value of K. The archipelago and subspecies origin for each sample is indicated below each bar (archipelago: V = Vanuatu, F = Fiji, SOL= Solomon Islands, RC = red-capped robin from Australia; subspecies: VA = *ambrynensis*, FK = *kelinschmidti*, FT = *taveunensis*, SoK = *kulambangrae*, SoP = *polymorpha*). (B) Species tree estimated in *BEAST using subspecies of Pacific robin as the a priori taxonomic units. (C) Divergence time estimates of the SOL and VFS lineages estimated by IMa2. (D) Migration rates (gene flow) between the SOL and VFS lineages estimated by IMa2.

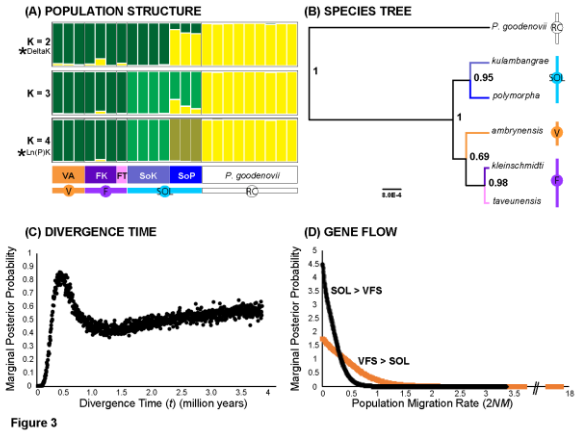

Figure 3

Figure 4. Variation in patterns of sexual dichromatism in the Pacific robin radiation. Boxplots depict the range of variation in plumage saturation of the back, crown and throat plumage patches in males (green) and females (yellow) from sexually dichromatic, elaborate monochromatic and dull monochromatic taxa. Differing russet-headed (r) and black-headed (b) males of *P. p. polymorpha* are plotted separately. The range in variation in plumage brightness and hue is shown in Supplementary Material Appendix 1, Fig. 4–5.

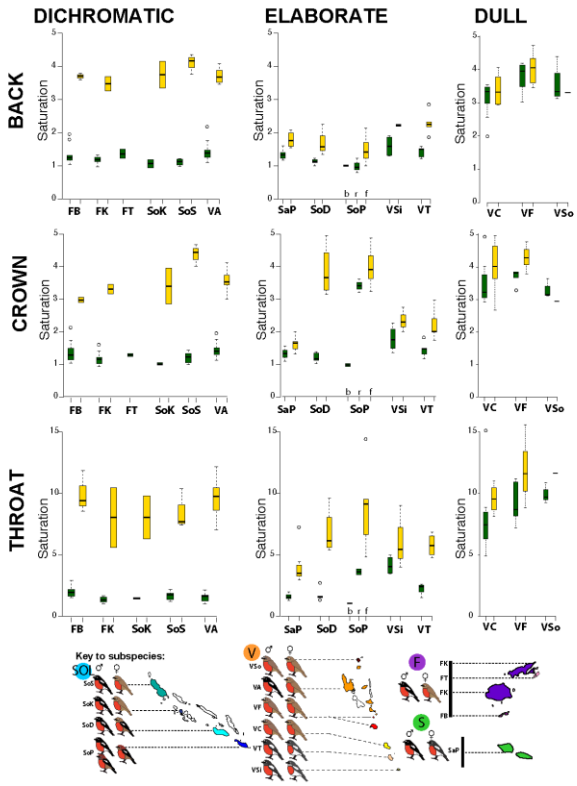

Fig 4.

Figure 5

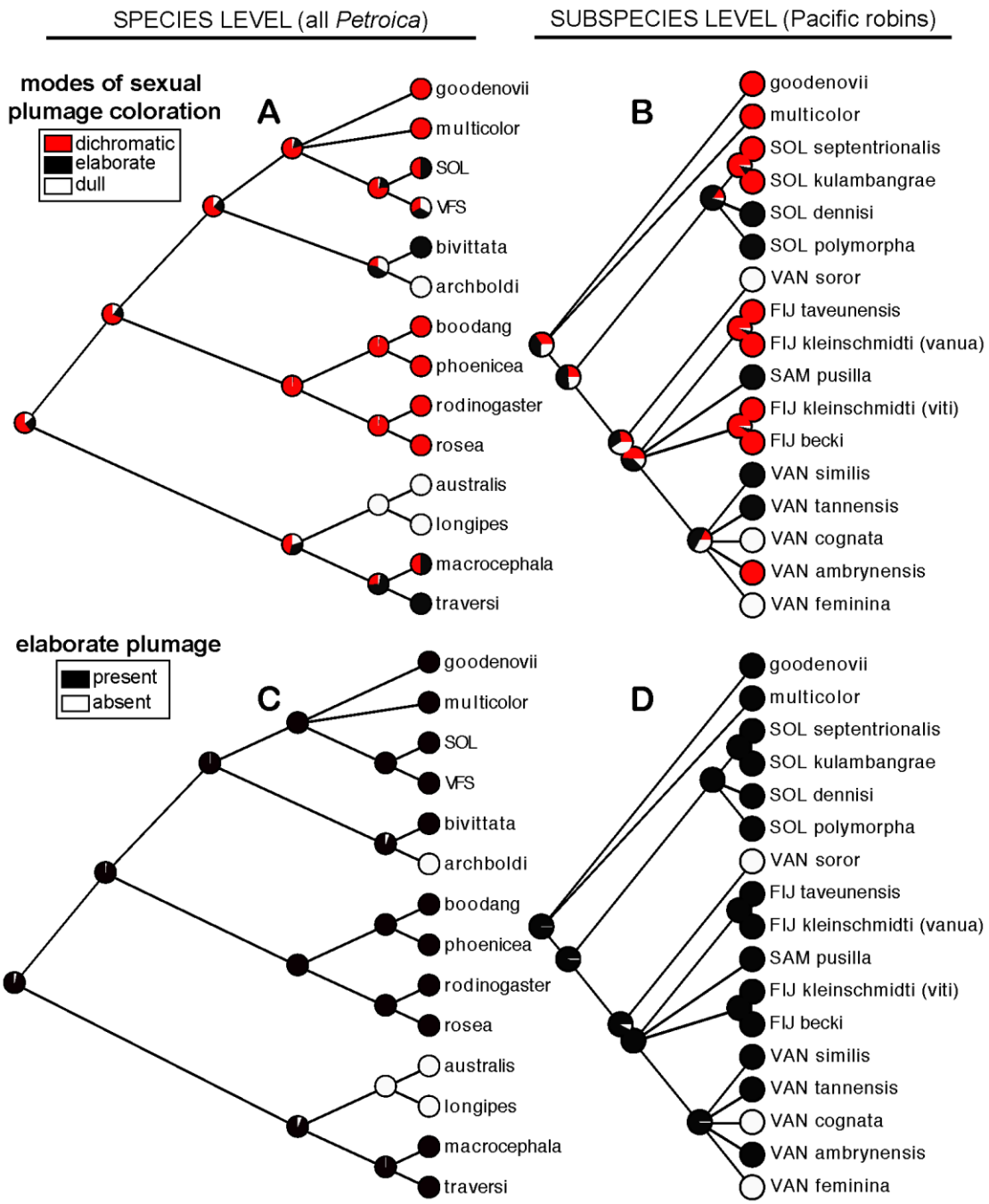

Figure 5.

Table Legends

Table 1. Measures of net mtDNA divergence between species/lineages, archipelagos/regions and subspecies within each archipelago in the southwest Pacific robin radiation. Values represent *Dxy* estimates expressed as a percentage. Abbreviations use the first three letters for subspecies names, the first letter for archipelagos and as follows – RC, red-capped robin; NI, Norfolk robin; SOL, Solomon Islands lineage; VFS, Vanuatu/Fiji/Samoa lineage.

Species / Lineages				
	RC	NI	SOL	VFS
RC	-	1.81	3.64	3.33
NI		-	3.71	3.11
SOL			-	3.85
VFS				-

Archipelagos / Regions						
	V	F	S	SOL	NI	RC
V	-	0.66	0.66	3.77	3.04	3.26
F		-	0.75	3.97	3.22	3.43
S			-	3.95	3.24	3.45

Solomon Islands				
	pol	kul	sep	den
pol	-	2.20	2.38	2.52
kul		-	1.81	1.92
sep			-	1.92
den				-

Vanuatu						
	sor	amb	fem	cog	tan	sim
sor	-	0.53	0.50	0.53	0.53	0.64
amb		-	0.18	0.21	0.21	0.32
fem			-	0.18	0.18	0.28
cog				-	0.21	0.32
tan					-	0.32
sim						-

Fiji						
	kle (viti)	kle (vanua)	bec	tav	bec & kle viti	tav & kle vanua
kle(viti)	-	0.85	0.32	0.80	-	-
kle(vanua)		-	0.96	0.16	-	-

bec		-	-	-
tav			-	-
bec&viti			-	0.87
tav&vanua				-
Samoa				
upolu vs savaii	0.21			

Table 2. MANOVA of morphometric variables calculated between Solomon, Vanuatu, Fiji and Samoa archipelagos, and among subspecies or islands within each archipelago. F statistic and probability (>F) is reported for each morphometric variable. The Pillai statistic (P), approximate F statistic (F) and probability (>F) is reported for the MANOVA across all variables. Significance is denoted in bold, and using symbol codes: '***' < 0.001, '**' < 0.01, '*' < 0.05.

		All Variables	Bill Length	Wing Length	Tail Length
Archipelagos	Males	**P=0.59088, aF=8.5844, p=1.631e-11*** **	**F=3.73, p=0.01355***	**F=22.157, p=3.416e-11*** **	**F=21.057, p=9.336e-11*** **
	Females	**P=0.56008, aF=4.2849, p=5.015e-05*** **	F=1.40, p=0.2514	**F=4.83, p=0.0047****	**F=13.046, p=1.428e-06*** **
Solomon subspecies	Males	**P=1.0938, aF=3.2518, p=0.003409****	F=0.50, p=0.6893	**F=10.561, p=0.0003717*** **	**F=12.471, p=0.0001478*** **
	Females	**P=1.4242, aF=4.8201, p=0.0001337*** **	F=0.3321, p=0.8022	**F=13.655, p=0.000112*** **	**F=8.3379, p=0.001447****
Vanuatu subspecies	Males	**P=1.0785, aF=4.4904, p=1.113e-06*** **	F=2.2487, p=0.06802 .	**F=2.8355, p=0.02773***	**F=12.219, p=3.205e-07*** **
	Females	**P=1.1294, aF=2.536, p=0.005251****	**F=2.9648, p=0.03533***	F=2.1969, p=0.09316 .	**F=3.1885, p=0.02692***
Fiji subspecies	Males	P=0.20486, aF=0.76079, p=0.6049	F=0.6489, p=0.5328	F=0.1031, p=0.9025	F=1.1122, p=0.3474
	Females	P=0.89793, aF=2.9323, p=0.3998	F=4.5542, p=0.1225	F=0.54, p=0.5157	F=2.6727, p=0.2006
Samoa islands	Males	P=0.11326, aF=0.59604, p=0.6279	F=0.0831, p=0.7768	F=1.7297, p=0.207	F=0.1, p=0.7559
	Females	P=0.5174, aF=1.4295, p=0.3584	F=4.9247, p=0.06828 .	F=0.04, p=0.8481	F=3.6214, p=0.1057

Table 3. MANOVA of plumage color variables calculated between subspecies within dichromatic, elaborate and dull plumage modes. For the elaborate monochromatic mode, males are divided into two tests: one excluding the red-headed morph of subspecies *polymorpha* from the Solomon Islands, and the other dividing red-headed and black-headed morphs of *polymorpha*. F statistic and probability (>F) is reported for each plumage colour variable. The Pillai statistic (P), approximate F statistic (F) and probability (>F) is reported for the MANOVA across all variables. Significance is denoted in bold, and using symbol codes: '***' < 0.001, '**' < 0.01, '*' < 0.05.

			Back			Crown			Throat		
		All Variables	Brightness	Saturation	Hue	Brightness	Saturation	Hue	Brightness	Saturation	Hue
Dichromatic	Males	**P=1.5725, aF=1.5834, p=0.04252***	F=0.7327, p=0.5777	**F=3.4403, p=0.02138***	F=2.3705, p=0.07752 .	F=0.2418, p=0.9121	**F=3.6991, p=0.01584***	F=1.7591, p=0.1663	F=1.4035, p=0.2595	**F=3.9197, p=0.01232 ***	F=2.444, p=0.07079
	Females	P=2.9133, aF=1.7873, p=0.06925 .	F=0.7788, p=0.5617	F=1.6198, p=0.238	F=0.8845, p=0.5045	**F=4.2053, p=0.02628***	**F=6.2339, p=0.007161****	**F=4, p=0.03051***	F=0.809, p=0.5448	F=0.7265, p=0.592	F=1.7734, p=0.2044
Elaborate Monochromatic	Males (all)	**P=3.1412, aF=3.9433, p=3.874e-09*****	F=1.2935, p=0.2982	**F=6.835, p=0.0003831*****	F=1.081, p=0.3948	**F=12.113, p=4.998e-06*****	**F=61.384, p=3.147e-13*****	F=1.2079, p=0.3343	**F=8.2415, p=0.0001039*****	**F=6.1114, p=0.0007906 *****	**F=5.1352, p=0.002244****
	Males (black)	**P=2.2924, aF=2.685, p=0.0001838*****	F=1.8466, p=0.1543	**F=6.2883, p=0.00143****	F=0.8183, p=0.5267	**F=5.7757, p=0.002276****	**F=7.5459, p=0.0004898*****	F=1.2901, p=0.303	**F=11.291, p=3.232e-05*****	**F=8.5761, p=0.0002175 *****	**F=5.828, p=0.002169****
	Females	**P=2.7959, aF=4.1278, p=3.894e-07*****	**F=22.639, p=2.309e-07*****	**F=4.2799, p=0.01092***	**F=10.479, p=7.926e-05*****	**F=35.25, p=4.891e-09*****	**F=47.801, p=2.964e-10*****	**F=18.695, p=1.105e-06*****	**F=13.9, p=1.083e-05*****	**F=7.2755, p=0.0007692 *****	**F=8.4457, p=0.0003171*****
Dull Monochromatic	Males	P=1.3788, aF=1.7262, p=0.1522	**F=3.7476, p=0.04972***	F=3.0103, p=0.08177 .	F=3.3328, p=0.06549 .	F=3.3973, p=0.0627 .	F=0.5713, p=0.5774	**F=3.7421, p=0.0499***	F=0.0599, p=0.9421	F=0.8673, p=0.4415	F=1.2665, p=0.3122
	Females	P=1.9243, aF=5.649, p=0.05267 .	F=2.0352, p=0.1865	F=3.674, p=0.06815 .	F=2.0594, p=0.1835	**F=7.8413, p=0.01067***	F=3.8155, p=0.06309 .	**F=6.0553, p=0.02157***	F=1.1257, p=0.3662	F=1.9296, p=0.2007	F=2.1287, p=0.175

Table 4. Standardized mean differences in the degree of sexual dichromatism of each subspecies for each plumage patch. Subspecies within each plumage mode are denoted by the first three letters of the archipelago and subspecies. For subspecies *polymorpha* (solpol), two tests are performed, one comparing females and red-headed morph males, and the other comparing females and the black-headed morph males. Variables with significantly different means between the sexes as determined from ANOVA are denoted in bold (Supplementary material Appendix 1, Table A3).

		Back			Crown			Throat		
		Brightness	Saturation	Hue	Brightness	Saturation	Hue	Brightness	Saturation	Hue
Dichromatic	solkul	**6.93**	**6.35**	**3.23**	**5.76**	**4.35**	**1.10**	**4.65**	**3.74**	**5.32**
	solsep	**18.11**	**16.35**	**5.94**	**7.64**	**13.38**	**2.17**	**7.94**	**7.34**	**5.06**
	vanamb	**5.30**	**8.58**	**3.83**	**7.65**	**9.28**	**3.20**	**7.63**	**7.49**	**6.31**
	fijbec	**7.44**	**10.91**	**4.70**	**9.42**	**6.63**	**0.50**	**20.74**	**7.83**	**9.17**
	fijkle	**10.85**	**13.79**	**4.75**	**5.74**	**13.68**	**2.48**	**4.48**	**5.02**	**6.24**
Elaborate Monochromatic	solden	0.69	**2.12**	0.59	**4.62**	**8.93**	**3.41**	**12.78**	**7.60**	**3.40**
	solpol (black M)	1.38	1.31	0.47	**7.33**	**5.56**	**3.13**	1.82	1.87	0.92
	solpol (red M)	0.02	1.38	1.05	**1.79**	1.13	**3.19**	**2.96**	**2.44**	**1.82**
	vansim	**3.26**	2.30	**4.39**	1.13	1.04	1.11	**6.55**	1.25	**5.78**
	vantan	**2.61**	**3.70**	0.09	**3.16**	1.94	0.18	**7.14**	**4.39**	**4.78**
	sampus	**1.65**	**2.59**	**1.27**	**1.24**	**1.83**	**1.26**	**4.45**	**3.00**	**3.18**
Dull Monochromatic	vancog	0.10	0.43	0.70	0.16	1.24	0.64	2.07	0.62	2.34
	vanfem	0.42	0.58	0.51	**1.82**	**1.92**	1.11	**2.04**	1.22	**1.92**
	vansor	2.00	0.62	0.14	0.68	**1.18**	0.70	**0.69**	2.00	**2.07**