2015-11-13

The Roles of Geographic Isolation and Gene Flow in the Diversification of the Rufous Fantail Rhipidura rufifrons

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THE ROLES OF GEOGRAPHIC ISOLATION AND GENE FLOW IN THE DIVERSIFICATION OF THE RUFOUS FANTAIL *RHIPIDURA RUFIFRONS*

By

Douglas E. Weidemann

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

December 2015

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
the requirements for the degree of
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WEIDEMANN, DOUGLAS E. (M.S., Biology)
The Roles of Geographic Isolation and Gene Flow in the Diversification of the Rufous Fantail *Rhipidura rufifrons* (December 2015)

Abstract of a thesis at the University of Miami.

Thesis supervised by Associate Professor J. Albert C. Uy.
No. of pages in text. (57)

Studies of divergence and speciation patterns in island systems have played an important role in the development and establishment of the allopatric speciation model. However, recent empirical support for divergence and speciation with gene flow means the importance of isolation for divergence in island systems needs to be re-examined. Here I explore the roles of geographic isolation and gene flow in the early stages of divergence of evolutionarily independent replicate populations of the Rufous Fantail *Rhipidura rufifrons* on satellite islands in southeastern Solomon Islands. These populations differ in the extent of morphological divergence from the main island, providing a unique opportunity to test between modes of divergence in an island system. Patterns of population structure, gene flow, and the evolutionary history of the system were determined from one mtDNA and five nuclear genetic markers. Two demographic factors, gene flow and divergence time, are closely associated with neutral genetic divergence and may explain the pattern of morphological divergence across the system. Additionally, extensive morphological divergence in this system is only occurring between islands experiencing little gene flow, providing support for the prevalence of allopatric divergence in island systems.

Acknowledgments

I would like to specially thank my advisor J. Albert C. Uy for his continued support, help, and guidance throughout this project. I would also like to thank the individuals who have been a part of my thesis committee, William Searcy, Leonel Sternberg, Jaime Chaves, and Alex Wilson, for their guidance and advice. I want to thank A. Graham, J. Sardell, E. Cooper, J. Waite, and K. McCracken for assisting me with various aspects of this project, and the rest of the members of the Uy and Searcy labs, S. Cowles, J. Hidalgo, W. Beckles, D. Baldassarre, L. Vargas, G. Leighton, and K. Rivera-Caceres, and A. Dubois for their helpful advice regarding this project. I also would like to thank the rest of the graduate students for their friendship and support.

Finally, I want to thank the people of Makira, Ugi, Santa Catalina, and Three Sisters in the Solomon Islands for their hospitality and allowing me to do field work on their islands. I also want to thank the local field assistants from the Solomon Islands who provided invaluable assistance with the field portion of this project.

Field work was conducted under permits provided by the Ministry of Education, Solomon Islands, and this project was approved by the Institutional Animal Care and Use Committee of the University of Miami, Coral Gables, Florida. Funding was provided by the Aresty Chair in Tropical Biology, Jay M. Savage Graduate Research Support Fund, and Kushlan Graduate Research Support Fund. J.A.C. Uy, D. Baldassarre, S. Cowles, and W. Beckles helped improve this thesis by providing valuable feedback.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

Chapter 1. Introduction

The production of biodiversity is an important question that remains a topic of ongoing research in biology (e.g., Gaston 2000, Butlin et al. 2012). One important source of biodiversity is new species which evolve through the process of speciation (e.g., Gaston 2000, Mittelbach et al. 2007). Speciation can be thought of in stages, with population divergence as the earliest stage (Mayr 1942, Coyne and Orr 2004, Price 2008). Historically, models of speciation can be divided into two main paradigms based on how populations diverge. In the allopatric speciation model, championed during the neo-Darwinian synthesis, geographic isolation is needed for divergence to proceed (Mayr 1942, Grant 2001, Price 2008). Without geographic isolation, gene flow will prevent the evolution of reproductive isolation and speciation due to its strong homogenizing effect, which can inhibit divergence between populations (Mayr 1942). Alternatively, in the speciation with gene flow model, which includes the extreme scenario of sympatric speciation, selection can cause divergence and reproductive isolation to evolve in spite of gene flow's homogenizing effects (e.g., Kondrashov and Kondrashov 1999, Coyne and Orr 2004, Feder et al. 2012). Thus in order to understand the speciation process it is important to understand the impact of gene flow on divergence, specifically whether or not gene flow strongly inhibits divergence making geographic isolation critical for divergence to proceed.

During the neo-Darwinian synthesis in the early and mid-twentieth century, studies of divergence and speciation patterns in islands helped establish the consensus position that nearly all speciation events occurred due to allopatric speciation (e.g., Mayr 1942, Grant 2001). Evidence from islands used to support the ubiquity of allopatric

speciation includes distribution patterns in which dispersal barriers, such as open ocean between islands, are present between the ranges of related species or incipient species (e.g., Mayr and Diamond 2001). Further, assessments of birds and *Anolis* lizards endemic to small islands (on which speciation events would likely include gene flow) failed to uncover any well-supported instances of speciation on individual islands and demonstrated that the endemic species were mainly generated by allopatric speciation between islands (Coyne and Price 2000, Losos and Schluter 2000).

Advances in genetics have produced new ways to study the evolutionary histories of speciation events and estimate gene flow between speciating taxa, allowing studies to better distinguish among speciation models (e.g., Barraclough and Nee 2001, Nielsen and Wakeley 2001, Bolnick and Fitzpatrick 2007). Also, new interest has developed in investigating the plausibility and importance of the speciation with gene flow model (e.g., Bolnick and Fitzpatrick 2007, Nosil 2008). These two factors have led to a number of recent studies supporting the speciation with gene flow model, including both theoretical models and experimental research that have found that disruptive selection is able to produce divergence, reproductive isolation, and in the case of theoretical models, speciation, with even large amounts of initial gene flow (e.g., Soans et al. 1974, Rice and Salt 1990, Rice and Hostert 1993, Kondrashov and Kondrashov 1999, Doebeli and Dieckmann 2003). Importantly, naturally occurring cases of divergence or speciation with gene flow are also now known and have been well-studied. For example in sticklebacks, selection has generated divergence between populations in several traits in spite of gene flow (e.g., Berner et al. 2009, Hendry et al. 2009). Some reproductive isolation has also evolved between various populations, suggesting that speciation is

proceeding in spite of gene flow (e.g., Berner et al. 2009). Other important examples that show similar patterns consistent with the speciation with gene flow model include *Timema* stick insects (e.g., Nosil 2007), *Rhagoletis* flies (Bush 1969, Filchak et al. 2000), crater lake cichlids (Schliewen et al. 2001, Barluenga et al. 2006), and *Gyrinophilus* cave salamanders (Niemiller et al. 2008). These studies indicate that gene flow may be likely during the early stages of divergence and speciation, a result contrary to the assertions of the proponents of the allopatric speciation model.

Similar evidence supporting the speciation with gene flow model is also beginning to mount in island systems. A number of island taxa show evidence consistent with selection producing divergence or speciation with gene flow such as *Howea* palms on Lord Howe Island (Savolainen et al. 2006), *Anolis* lizards on Dominica (Stenson et al. 2002), Darwin's Finches (e.g., Petren et al. 2005, Huber et al. 2007, de León et al. 2010, Galligan et al. 2012), *Monarcha* flycatchers in Melanesia (Uy et al. 2009b), and *Cyanistes* tits on Corsica (e.g., Blondel et al. 2006). Additionally, examples of diversification with gene flow in island systems span a variety of geographic scenarios, including sympatric speciation or divergence on small islands (e.g., Savolainen et al. 2006, Friesen et al. 2007), parapatric divergence between populations on the same island (e.g., Stenson et al. 2002, Blondel et al. 2006, Galligan et al. 2012), and even divergence with gene flow between populations on separate islands (e.g., Petren et al. 2005, Uy et al. 2009b, Clegg and Phillimore 2010). These examples are in contrast to island systems' previous role as an important source of evidence for the ubiquity of the allopatric speciation model (e.g., Mayr 1942, Grant 2001) and point toward the possibility that

speciation with gene flow may be an important and even widespread mode of speciation in island systems.

With increasing evidence for divergence and speciation with gene flow, the importance of isolation for morphological divergence in island systems needs to be re-examined. Ideal island systems for testing the importance of geographic isolation should contain taxa undergoing divergence among islands that are close enough to each other that allopatry cannot be automatically assumed. To effectively test if isolation is important, the study should include replicate island populations likely experiencing various levels of isolation and divergence (e.g., Hendry et al. 2001, Hendry and Taylor 2004). Divergence in morphological traits despite ongoing gene flow between populations would support the speciation with gene flow model. On the other hand, two results would support the allopatric speciation model. First, finding substantial morphological divergence, but very little or no gene flow between populations would show that the system is diverging in isolation. Second, finding lower morphological divergence associated with more gene flow would support the importance of isolation for divergence (e.g., Calsbeek and Smith 2003, Hendry and Taylor 2004). However, such patterns must be interpreted carefully. Divergence times must be considered because younger populations will not have had time to diverge as much as older populations (Kinnison and Hendry 2001, Price 2008), which can lead to a false relationship of morphological divergence with gene flow if gene flow and divergence times are correlated. Thus these types of studies should simultaneously estimate both divergence times and levels of contemporary gene flow.

Several recent studies of archipelago systems in which gene flow varies among islands have investigated whether or not the amount of morphological divergence varies with gene flow or gene flow-driven genetic structure (Calsbeek and Smith 2003, Petren et al. 2005, Clegg and Phillimore 2010). However, only one of those studies found a relationship between the two consistent with gene flow inhibiting divergence (Calsbeek and Smith 2003). Thus these studies also suggest that isolation may not always be necessary for divergence in island systems.

Here I explore the relative roles of geographic isolation and gene flow in the early stages of divergence of an island system where populations differ in the extent of divergence in morphological traits. The Rufous Fantail *Rhipidura rufifrons* is a wide-ranging and highly variable species of Australasian flycatcher (Mayr 1931, Mayr and Moynihan 1946, Boles 2006). It has an extensive range including Australia and several Pacific archipelagos, extending from Indonesia to Melanesia and Micronesia (Boles 2006). More importantly, substantial morphological divergence, including differences in plumage patterns, size, and shape, has evolved between adjacent populations occurring on different islands (Mayr and Moynihan 1946). This high level of diversity provides many opportunities to examine the evolution of island populations including how morphological divergence occurs between islands (Mayr and Moynihan 1946).

More specifically, I focus on a complex of three subspecies found in southeastern Solomon Islands that differ in the extent of morphological divergence and are separated by minor ocean barriers. In this system, near the main island of Makira are two groups of small satellite islands containing potentially evolutionarily independent replicate populations that have diverged morphologically from the main island to varying degrees

(Mayr 1931, Mayr and Diamond 2001, Dutson 2011; Fig. 1). Island populations southeast of Makira show almost no morphological divergence from Makira, while those north of Makira show extensive divergence in morphology (Mayr 1931, Mayr and Diamond 2001, Dutson 2011; Fig. 1). Both island groups are within 10 km of Makira, effectively controlling for geographic distance.

I investigated the relationship among gene flow, evolutionary history, and population divergence in order to test key predictions of the two speciation models, allopatric speciation and speciation with gene flow, and understand the evolution of morphological divergence in this system (Table 1). If geographic barriers are critical to divergence (i.e., the allopatric speciation model), only the morphologically undifferentiated satellite population southeast of Makira should show evidence for gene flow with the main island, while the morphologically divergent satellite population north of Makira should be isolated. Isolation of both satellite populations would also be consistent with the allopatric speciation model but the undifferentiated population should be younger than the divergent population. Finally, if divergence is occurring under the speciation with gene flow model, both satellite populations should experience ongoing gene flow with the main island. This pattern would suggest that strong selection is keeping the northern population distinct in morphology despite gene flow. In order to test these predictions, I measured gene flow and population structure among islands using presumably neutral mitochondrial and nuclear genetic markers.

Chapter 2. Methods

Study System

The southeastern Solomon Islands consist of the large main island of Makira and two main groups of small, nearby satellite islands (< 10 km from Makira): Santa Ana and Santa Catalina southeast of Makira, and Ugi and Three Sisters north of Makira (Fig. 1). Despite late Pleistocene variation in sea levels, ocean barriers have kept Makira and the satellite islands isolated for some time (possibly always) (Diamond and Mayr 1976, Mayr and Diamond 2001). Thus any recent dispersal between island groups would involve crossing open water for less than 10 km.

The southeastern Solomon Islands contain three endemic subspecies of Rufous Fantail (Mayr 1931, Mayr and Diamond 2001). *R. r. russata* is endemic to the main island of Makira, *R. r. kuperi* is endemic to the southeastern islands Santa Ana and Santa Catalina, and *R. r. ugiensis* is endemic to the northern islands Ugi and Three Sisters (Mayr 1931, Mayr and Diamond 2001, J.A.C. Uy, pers. comm.). *R. r. russata* has mostly rufous upperparts and whitish underparts, with a black band separating the throat and chest (Mayr 1931, Dutson 2011). *R. r. kuperi* is nearly identical to *R. r. russata*, with only small differences in back and hindneck color and a larger forehead patch (Mayr 1931, Dutson 2011). In contrast, *R. r. ugiensis* is highly divergent in plumage, showing extensive black on the throat, and is reported to be slightly larger than the other subspecies (Mayr 1931, Mayr and Moynihan 1946, Dutson 2011; Fig. 1).

Sampling

During 2013 and 2014, Rufous Fantails were captured using mist nets from locations in Makira-Ulawa Province, Solomon Islands, corresponding to the three subspecies in the study system: Santa Catalina (n = 11; *R. r. kuperi*), Ugi (n = 30; *R. r. ugiensis*), and two locations on the main island of Makira (*R. r. russata*), central Makira (n = 13) and Star Harbor in eastern Makira (n = 4). To make sure that individuals were only sampled once, each individual was banded with a numbered metal band and a unique pattern of colored bands. Tail length and unflattened wing chord were measured to the nearest mm with a wing ruler, while head-bill length from the rear of the head to the bill tip and tarsus length from the intertarsal joint to the base of the toes were measured with digital calipers. Both left and right wings and tarsi were measured and the values averaged. Next, a Pesola® spring scale (Pesola AG, Switzerland) was used to measure the bird's mass to the nearest 0.5 g. Finally, two plumage patches that vary within the study system were also measured with a ruler to the nearest mm. The extent of white feathers on the chin and throat was measured by holding the bird's head back with the bill parallel to the main body axis, stretching out the throat. The length of the white patch was then measured along the center of the body from the base of the bill to the first row of black feathers. The extent of black pigment on the two central rectrices was also measured along the rachis of each feather and the two values averaged for each bird. Finally, a blood sample was collected from the wing vein of each individual and placed in 1 ml of lysis buffer. Samples were transported to the University of Miami, Coral Gables, FL, and stored at -20° C.

Two-sample t-tests with unequal variance were used to test for significant differences in wing chord, tail length, tarsus length, head-bill length, and mass among the three islands. Additionally, a principal component analysis (PCA) was performed on all six measurements (*R. r. kuperi* was excluded due to small sample size) and two-sample t-tests were used to test for differences in the first two principal components between *R. r. russata* and *R. r. ugiensis*. Finally, due to a lack of normality, Wilcoxon rank sum tests were used to test for significant differences in plumage patch measurements. All statistics were performed in R with the package "stats" (R Core Team 2013) and a Holm-Bonferroni correction (Holm 1979) was applied to the p-values from each set of tests to correct for multiple comparisons.

Sequencing

DNA was extracted from blood samples following the manufacturer's protocol using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). The protocol was modified to improve DNA yield by adding overnight incubation and the final spin step was repeated using half the product each time.

One mitochondrial marker and five nuclear introns were sequenced for each individual. PCR was used to amplify each marker using previously published primers. PCR master mixes and PCR amplification protocols were first optimized to ensure adequate and clean amplification before amplifying all the samples. I amplified the mitochondrial marker nicotinamide adenine dinucleotide dehydrogenase subunit 2 (*ND2*) with the previously developed primers L5215 and H1064 (Hackett 1996, Drovetski et al. 2004). For nuclear introns, I amplified the following: *15246* (Backström et al. 2008),

beta-fibrinogen intron 5 (*BFIB5*; Marini et al. 2002), myoglobin intron 2 (*MYO*; Slade et al. 1993, Heslewood et al. 1998), rhodopsin intron 1 (*RHO*; Primmer et al. 2002), and transforming growth factor β2 intron 5 (*TGFB2*; Primmer et al. 2002).

PCR amplification was performed on either a Bio-Rad T100™ or MyCycler™ thermal cycler (Bio-Rad Laboratories Headquarters, Hercules, CA). PCR product was checked for contamination and proper amplification by running 4 µl through a 1% agarose gel. Next, PCR product was cleaned by adding 1 µl of ExoSAP-IT® (Affymetrix, Santa Clara, CA) to every 10 µl of PCR product and running on a thermal cycler following the ExoSAP-IT® protocol. The sequencing reaction then used this cleaned PCR product and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Austin, TX). Finally, sequencing product was filtered through Sephadex columns (GE Healthcare, Pittsburgh, PA) before sequencing. Sequencing included both forward and backward reads, and was performed in the Molecular Core facility of the Department of Biology, University of Miami, FL.

Sequence Alignment and Summary Statistics

Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI) was used to align sequences and correct miscalled base pairs. For each sequenced marker, sequences were trimmed to a uniform length and heterozygote SNP sites (nuclear markers only) were coded as ambiguous before export. Exported sequences were imported into DnaSP 5.10 (Librado and Rozas 2009) and each marker was tested for Hardy-Weinberg equilibrium. Also, for the diploid nuclear markers, DnaSP was used to determine haplotypes for each individual using the default settings for PHASE (Stephens et al. 2001, Stephens and

Donnelly 2003), and examine the markers for recombination with the four-gamete test (Hudson and Kaplan 1985). Finally, number of haplotypes and nucleotide diversity (π) for each marker were determined for each population using ARLEQUIN 3.5 (Excoffier et al. 2005).

Population Structure

Population structure was measured using multiple methods including haplotype networks, STRUCTURE analysis, and population genetic statistics of population differentiation. Low levels of population structure can be caused by ongoing gene flow and recent divergence, while high levels of population structure require isolation (e.g., Halliburton 2004). To examine mitochondrial population structure, I produced a haplotype network in TCS 1.21 (Clement et al. 2000) through statistical parsimony methods including a connection limit of 95%.

A STRUCTURE analysis (Pritchard et al. 2000, Hubisz et al. 2009) was performed using the five nuclear intron markers to determine the number of genetic populations present and their composition as indicated by the nuclear markers. The program STRUCTURE 2.3.4 was run using the admixture and LOCPRIOR models (Pritchard et al. 2000, Hubisz et al. 2009). The admixture model permits hybridization, while the LOCPRIOR model increases model power by incorporating geographic information as a prior, yet does not cause STRUCTURE to detect nonexistent population structure (Pritchard et al. 2000, Hubisz et al. 2009). To achieve adequate mixing, the parameter ALPHAPROPSD was set at 0.1. Each run consisted of 100,000 burn-in steps followed by 1,000,000 steps, while all other settings kept the default options. To

determine the number of genetic populations present, 10 runs were conducted for each possible number of populations tested, which ranged from one to six. The outputs from each run were collected and uploaded to STRUCTURE HARVESTER, which uses the likelihood values for all of the runs to identify the most likely number of genetic populations (Earl 2012).

Once the number of populations was determined, population composition was examined using only those runs with the correct number of populations. The output from STRUCTURE HARVESTER was run through the program CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and then DISTRUCT 1.1 (Rosenberg 2004) in order to determine which population each individual belongs to.

F_{st} and D_{xy} statistics were used to measure the degree of divergence between island populations. F_{st} measures the extent of divergence based on patterns of allele frequencies (Halliburton 2004), while D_{xy} measures the average genetic distance per base pair between haplotypes from the two populations (Nei 1987), thus giving two different measures of divergence. F_{st} values between islands were calculated in ARLEQUIN and D_{xy} values were calculated in DnaSP.

Phylogenetic Trees

In order to uncover the evolutionary history of the system, several phylogenetic trees were estimated using both the mtDNA and nuclear markers. A Bayesian phylogeny of *ND2* haplotypes was produced in BEAST v1.8.1 (Drummond et al. 2012) from all the unique *ND2* haplotypes sequenced in this study plus additional *ND2* sequences representing all other Rufous Fantail subspecies in the Solomon Islands archipelago

except for *R. r. brunea* (endemic to Malaita) which were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The tree was rooted with additional sequences from two closely related fantail species, *Rhipidura dryas* and *R. teysmanni*, also acquired from GenBank. PartitionFinder (Lanfear et al. 2012) was used with the BIC to determine the best available substitution model for the sequences: HKY + I. The Yule Process was chosen for the tree prior and a strict molecular evolutionary clock was used with a substitution rate of 2.1% per site per lineage per million years. This rate was chosen based on a 2.0–2.2% substitution rate for *ND2* estimated by Arbogast et al. (2006) from divergence times of mockingbirds (Mimidae) on islands in the eastern Pacific with geologically determined ages, which has since been used to help estimate divergence times of several additional island passerines (e.g., Moyle et al. 2009, Uy et al. 2009a).

BEAST was run for 100 million steps with a burn-in of 10% and results were recorded every 1000 steps. TRACER v1.6 (Rambaut and Drummond 2013) was used to examine the results for stationarity, and sufficient burn-in times and Effective Sample Size (ESS) values (> 200). Finally, TreeAnnotator v1.8.1 (BEAST package) and FigTree v1.4 (Rambaut 2006) were used to examine the maximum clade credibility tree.

Phylogenies were also created in *BEAST (Heled and Drummond 2010), a program based on a Bayesian multispecies coalescent model that produces population level species trees by utilizing multiple markers. This method improves the ability to determine phylogenetic relationships among populations when lineage sorting is not complete, as well as removes the need to concatenate multiple markers which may cause the tree to show incorrect relationships among taxa (Kubatko and Degnan 2007, Degnan and Rosenberg 2009, Heled and Drummond 2010). However, because gene flow violates

*BEAST's assumptions, the presence of gene flow may affect the tree topology and divergence times (Leaché et al. 2014).

The *BEAST trees included the three study populations, plus two outgroups using sequences downloaded from GenBank: another Solomon Islands Rufous Fantail subspecies *R. r. rufofronta* (1 individual) and a close relative of Rhipiduridae *Chaetorhynchus papuensis* (1 individual). The first tree consisted of only the nuclear markers *BFIB*, *MYO*, and *TGFB2* (the other nuclear markers were excluded due to lack of available outgroup sequences from GenBank) and the second included these three nuclear markers plus the mtDNA marker *ND2*. *TGFB2* was trimmed to remove recombination sites and PartitionFinder was used with the BIC to determine the best available substitution models, resulting in the following choice of models: HKY for *BFIB*, *MYO*, and *TGFB2*, and HKY + I for *ND2*. The tree prior was set to Species Tree: Yule Process and the population size prior was set to Piecewise Linear and Constant Root. A strict molecular clock was used in both trees, and for the tree that included mtDNA, the same substitution rate of 2.1% per site per lineage per million years was applied to the *ND2* locus. *BEAST was then run for 100 million steps for the nuclear marker only tree and for 500 million steps for the mtDNA and nuclear combined tree, with a burn-in of 10% and results recorded every 1000 steps. *BEAST output was again examined in TRACER for stationarity, and sufficient burn-in times and ESS values, while TreeAnnotator and FigTree were used to examine the maximum clade credibility tree.

Isolation with Migration Model

In order to simultaneously estimate migration rates, relative population sizes, and divergence times, the coalescent model IMa2 (Hey 2010) was run using the mtDNA and five nuclear markers from the three study populations with the *TGFB2* sequences first trimmed to remove recombination sites. The Infinite Sites substitution model was applied to the nuclear markers while the HKY model was applied to the mtDNA *ND2* marker. To enable the conversion of coalescent time units into years, the substitution rate of 2.1% per site per lineage per million years was again applied to the *ND2* marker.

Uniform priors were used for migration rates, divergence times, and the population size parameter θ ($4N_eu$), and their ranges were first optimized through preliminary runs with various combinations of priors including priors with much larger ranges than the final priors. A geometric heating scheme and 200 chains were used to ensure adequate mixing. Once the optimal priors were determined, the model was run for 5,000,000 steps preceded by a burnin-in of 2,000,000 steps. Genealogies were recorded every 100 steps for a total of 50,000 genealogies per run and the results were checked for stationarity and proper mixing. In addition to producing estimates of divergence times, migration rates, and θ, the program was also used to estimate the number of effective gene migrants per generation between each pair of populations.

Chapter 3. Results

Morphological Analysis

Of the five size measurements taken, individuals from the satellite island population *R. r. ugiensis* were significantly larger than individuals from the main island population *R. r. russata* for tarsus length, head-bill length, and mass (Tables 2 and 3). In contrast, individuals from the second satellite population *R. r. kuperi* did not differ significantly from either the main island population *R. r. russata* or the first satellite population *R. r. ugiensis* in any of the body size measurements or mass (Tables 2 and 3).

The PCA produced a first principal component that explained 49% of the variance and characterized overall body size since it was positively loaded with all body size measurements and mass (Table 4). The second principal component explained an additional 20% of the variance and was positively loaded with wing chord and tail length, and negatively loaded with tarsus length, head-bill length, and mass (Table 4). Satellite island population *R. r. ugiensis* individuals had significantly higher values than main island population *R. r. russata* individuals along the first principal component axis, demonstrating that *R. r. ugiensis* individuals had larger overall body size (Table 4, Fig. 2). The two populations did not differ significantly along the second principal component axis (Table 4).

R. r. ugiensis also differed significantly from both the main island population and other satellite island population in terms of plumage patch size, showing a smaller white patch or all black throat and chin ($W = 344$, $P < 0.001$; and $W = 226$, $P < 0.001$; respectively) and more extensive black on the central rectrices ($W = 0$, $P < 0.001$; $W = 2$, $P < 0.001$; respectively; Fig. 3). The other satellite population *R. r. kuperi* did not differ

significantly from the main island population in either measurements (throat patch: $W = 56$, $P = 0.56$; central rectrix patch: $W = 22$, $P = 0.08$; Fig. 3).

Sequencing Results

The five nuclear markers and one mitochondrial marker were successfully sequenced for all samples except for *15246* which could not be sequenced for two *R. r. kuperi* and one *R. r. russata* samples (Table 5). None of the markers differed significantly from Hardy-Weinberg equilibrium after a Holm-Bonferroni correction was used to correct for multiple tests. For the diploid nuclear markers, haplotypes were determined with high probability (> 0.8) for nearly all sequences (95–100% of individuals per marker; Table 5), while only one marker *TGFB2* showed evidence of recombination. The number of variable (SNP) sites in each marker varied from three in *15246* to 30 in *ND2*, while nucleotide diversity varied from 0.0 to 0.0083 (Table 5).

Mitochondrial Structure

The mtDNA *ND2* marker showed a strong pattern of genetic structure between the northern satellite population, and the mainland and southeastern populations. In the *ND2* haplotype network, all but two individuals from the northern satellite population *R. r. ugiensis* (28 out of 30, 93%) had a single highly divergent haplotype separated from the nearest haplotype by eight substitutions (0.99% divergence; Fig. 4). In contrast, the three haplotypes from the southeastern *R. r. kuperi* population were separated from main island *R. r. russata* haplotypes by at most three substitutions (0.4% divergence) and one haplotype found in four *R. r. kuperi* individuals was shared with one main island

individual. While all *ND2* F_{st} values were significantly greater than zero, indicating that mtDNA structure was present between all pairs of populations, both F_{st} and D_{xy} values were much higher between *R. r. ugiensis* and the other two populations, than between the main island and *R. r. kuperi* (Table 6).

Nuclear Structure

The nuclear markers showed less overall structure and less clear patterns of divergence than the mtDNA. Both average F_{st} and D_{xy} values between the satellite populations *R. r. ugiensis* and *R. r. kuperi* were relatively large, indicating structure between the two (Table 6). In contrast, F_{st} and D_{xy} values between the main island population and both satellite islands were very low on average, suggesting little divergence of either satellite population from the main island (Table 6). However, the results of the STRUCTURE analysis, which combined all five nuclear makers, showed a stronger pattern of genetic structure with two distinct genetic populations present in the system. The first genetic population consisted of all individuals from the northern satellite population *R. r. ugiensis*, while birds from the main island population *R. r. russata* generally clustered with the southeastern satellite population *R. r. kuperi* in the second genetic population, showing only low probabilities of clustering with *R. r. ugiensis* (Fig. 5).

Phylogeny

All phylogenetic trees showed high posterior support for a monophyletic clade in southeastern Solomon Islands consisting of the three study populations, *R. r. russata*, *R.*

r. kuperi, and *R. r. ugiensis*, and distinct from other populations in the Solomon Islands archipelago (Figs. 6 and 7). In contrast, the phylogeny within the southeastern Solomon clade is poorly resolved. In the *ND2* BEAST tree, none of the populations' haplotypes form a monophyletic group (Fig. 6). The first lineage to split off from the base of the clade is the main haplotype from the satellite population *R. r. ugiensis*, suggesting *R. r. ugiensis* might have diverged first (Fig. 6). However, monophyly for the rest of the clade is not well supported (posterior probability = 0.58) and the second (although rare) *R. r. ugiensis* haplotype is nested within the haplotypes of the main island population *R. r. russata* near the tip of the tree (Fig. 6). Likewise, the three haplotypes from the satellite population *R. r. kuperi* group with several haplotypes from the main island population (Fig. 6).

The *BEAST trees are also poorly resolved within the southeastern Solomon clade (Fig. 7). The tree consisting of only nuclear markers places the main island population *R. r. russata* and the satellite population *R. r. kuperi* together as sister taxa similar to the mtDNA BEAST tree, but also with the low posterior probability 0.65 (Fig. 7). However, the combined mtDNA and nuclear marker tree suggests a different topology in which the two satellite populations *R. r. ugiensis* and *R. r. kuperi* are sister taxa, but with an even lower posterior probability of 0.43 (Fig. 7).

Divergence Times

Based on the strict molecular clock and substitution rate of 2.1% per site per lineage per million years, the *ND2* BEAST tree suggests the most recent common ancestor of the southeastern Solomon clade and other Solomon Island archipelago

populations is estimated to have occurred 674,000 years ago (95% highest posterior density interval [HPD] = 477,000–909,000). Although including fewer taxa, the combined mtDNA and nuclear *BEAST tree estimates the most recent common ancestor of the southeastern Solomon clade and the adjacent island *R. r. rufofronta* population occurred 466,000 years ago (95% HPD = 206,000–752,000). The most recent common ancestor of the southeastern Solomon clade is estimated in the *ND2* BEAST tree to have occurred 305,000 years ago (95% HPD = 198,000–434,000) while the *BEAST estimate is much younger at 43,400 years ago (95% HPD = 17,600–78,200).

IMa2 Results

The IMa2 results estimate that *R. r. ugiensis* diverged from the ancestor of the other two populations 213,000 years ago (95% HPD = 22,000–1,333,000 years), while *R. r. kuperi* diverged from *R. r. russata* much more recently, only 15,400 years ago (95% HPD = 0–797,000 years; Fig. 8). As expected based on island size, estimates of θ were much higher for the main island population *R. r. russata* and its ancestral populations than either of the two satellite populations, suggesting *R. r. russata* has a much larger effective population size (Fig. 8).

Only two of the eight migration rates had 95% HPD intervals that did not include zero, indicating statistically significant migration: from *R. r. russata* to *R. r. kuperi* (m = 14.6, 95% HPD = 0.2–90.2), and from *R. r. russata* to *R. r. ugiensis* (m = 100.0, 95% HPD = 19.0–100.0; Fig. 8). Although not statistically significant, the shape of the posterior density curve suggests migration may also be occurring from *R. r. kuperi* to *R.*

r. russata, and potentially between *R. r. ugiensis* and the ancestor of the other two populations (Fig. 8).

When converted into $2N_em$ values, the estimated number of effective gene migrants per generation from *R. r. kuperi* to *R. r. russata* was 3.12 (95% HPD = 0.0–460.4) and 1.15 (95% HPD = 0.0–3.98) from *R. r. russata* to *R. r. kuperi* (Fig. 9). Estimates were lower between *R. r. ugiensis* and *R. r. russata*: 0.90 (95% HPD = 0.22–2.10) from *R. r. russata* to *R. r. ugiensis* and 0.62 (95% HPD = 0.0–190.5) from *R. r. ugiensis* to *R. r. russata*; and even lower between *R. r. ugiensis* and the ancestral population (0.62 [95% HPD = 0.0–677.8] from *R. r. ugiensis* to the ancestral population and 0.06 [95% HPD = 0.0–1.28] from the ancestral population to *R. r. ugiensis*; Fig. 9).

Chapter 4. Discussion

Morphology and Genetic Structure

Morphological and plumage measurements confirm the presence of both highly divergent and poorly differentiated satellite island populations in southeastern Solomon Islands. These measurements show that the satellite population *R. r. ugiensis* on Ugi is highly divergent from *R. r. russata* on the main island of Makira and the other satellite population *R. r. kuperi* on Santa Catalina, consistent with previous studies (Mayr 1931, Mayr and Moynihan 1946). Both the PCA and tests of individual size measurements (three of the five measurements showed a significant difference) demonstrate that *R. r. ugiensis* individuals from the northern island of Ugi are larger than individuals in the main island population. Analyses of the size of two plumage patches also showed that *R. r. ugiensis* differed strongly in plumage pattern, having significantly more extensive black plumage on the throat and tail than either of the other two populations. In contrast, the results are ambiguous regarding whether or not the second satellite population *R. r. kuperi* has diverged in size: *R. r. kuperi* did not differ significantly in any of the body size measurements from either of the other populations and the sample size was too small to include this population in the PCA. However, previous papers that describe the morphology of this system make no mention of any size differences between *R. r. kuperi* and the main island population *R. r. russata* (Mayr 1931, Mayr and Moynihan 1946) with Mayr and Moynihan (1946) describing them both as small while categorizing *R. r. ugiensis* as medium-sized, suggesting there may have been little divergence, if any, between *R. r. kuperi* and the main island population. Finally, *R. r. kuperi* failed to differ significantly from the main island population in either of the patch size measurements,

which is not surprising given that *R. r. kuperi* has been reported to only show subtle differences in forehead, hindneck, and back plumage, and not in tail or throat pattern (Mayr 1931, Mayr and Moynihan 1946, Dutson 2011).

The patterns of neutral genetic structure closely follow the patterns of color and size divergence within the study system. Of the two satellite island populations, the morphologically divergent *R. r. ugiensis* to the north generally shows greater neutral genetic structure and divergence between it and the main island population *R. r. russata*. F_{st} and D_{xy} values for *ND2* are relatively high and most *R. r. ugiensis* individuals have the mtDNA haplotype that is by far the most divergent haplotype in the system. While average nuclear F_{st} and D_{xy} values are low and similar to those between the main island population and the other satellite population *R. r. kuperi*, when nuclear loci are combined in the STRUCTURE analysis, *R. r. ugiensis* emerges as the most distinct genetic population.

While *R. r. kuperi* is consistently distinct from *R. r. ugiensis*, this weakly morphologically differentiated satellite population shows much less genetic structure and divergence from the main island population *R. r. russata*. Although the *ND2* F_{st} value between *R. r. kuperi* and *R. r. russata* is significantly different from zero, the F_{st} and D_{xy} values are much lower than those between the other satellite population *R. r. ugiensis* and the main island. This can be visualized in the haplotype network where the *R. r. kuperi* haplotypes are less divergent from the main island haplotypes and one of the haplotypes is also shared with a main island individual. Finally, like *R. r. ugiensis*, nuclear F_{st} and D_{xy} values between *R. r. kuperi* and the main island are low on average. However, in the STRUCTURE analysis, *R. r. kuperi* does not form its own distinct genetic population as

R. r. ugiensis does. Instead, it groups together with the main island population in a single genetic population, with the only difference that *R. r. kuperi* individuals have almost zero probability of belonging to the *R. r. ugiensis* genetic population, while main island individuals all have a low probability of grouping with *R. r. ugiensis*.

Phylogenetic History

The three study populations form a monophyletic clade distinct from other Solomon Islands archipelago populations, confirming that the three study populations all evolved from a single common ancestor (Figs. 6 and 7). On the other hand, the phylogeny within the southeastern Solomon clade was not well resolved for several reasons. First, two of the three trees, the *ND2* BEAST tree and the nuclear only *BEAST tree, resulted in a tree in which *R. r. russata* and *R. r. kuperi* are sister taxa, while the mtDNA and nuclear combined *BEAST tree suggested an alternative topology in which *R. r. kuperi* and *R. r. ugiensis* were sister taxa. Second, the topologies of all the trees had low posterior support meaning there is uncertainty within each tree in the chosen topology. Finally, the pattern in the *ND2* BEAST tree was further obscured by a lack of monophyly for any of the study populations.

However, despite this uncertainty, the overall results suggest the most likely topology is the one in which the satellite population *R. r. ugiensis* diverges first, followed by the splitting of the main island population *R. r. russata* and the second satellite population *R. r. kuperi*, and this tree was the one used in the IMa2 analysis. This topology is supported, although weakly, by two of the three trees. Furthermore, some of the discrepancies can be explained by the limited gene flow occurring within the study

system. While *BEAST is capable of incorporating incomplete lineage sorting which can also obscure phylogenies, gene flow violates the assumptions of the *BEAST model (Heled and Drummond 2010) and simulations have shown that the presence of slight gene flow can cause populations to appear more closely related than they really are, often negatively affecting the topology when gene flow is between non-sister taxa (Leaché et al. 2014). This effect may explain the discrepancies among the phylogenetic trees. The two *BEAST trees differ in the presence of the mtDNA *ND2* marker, which appears to show a gene flow event between two non-sister taxa *R. r. ugiensis* and *R. r. russata*. This may explain the unusual topology in the *BEAST tree that uses both mtDNA and nuclear markers, which is not supported by either the nuclear only *BEAST tree or the *ND2* BEAST tree.

Gene Flow, Divergence Time, and Genetic Structure

The results of this study demonstrate that the satellite island population *R. r. ugiensis* to the north is both older and exhibits limited gene flow with the main island population *R. r. russata*. In contrast, the southeastern satellite island population of *R. r. kuperi* is younger and there is substantial gene flow between it and the main island population. The high levels of genetic structure present between *R. r. ugiensis* and *R. r. russata* from both the STRUCTURE analysis (nuclear markers) and mtDNA structure analyses are consistent with very little or no gene flow between these two. In the STRUCTURE results, all *R. r. ugiensis* individuals have a very high probability of belonging to a unique genetic population, a pattern consistent with a lack of recent gene flow. Furthermore, the high F_{st} and D_{xy} values and lack of any shared mtDNA haplotypes

also support the lack of substantial gene flow between *R. r. ugiensis* and *R. r. russata*. However, other evidence suggests that gene flow is non-zero. The IMa2 model found significant levels of contemporary gene flow from the main island *R. r. russata* to *R. r. ugiensis* with an estimate of 0.90 effective gene migrants per generation. Additionally, a rare haplotype found in *R. r. ugiensis* clusters with other *R. r. russata* haplotypes and suggests some previous gene flow into *R. r. ugiensis*. Finally, *R. r. russata* individuals in the STRUCTURE analysis show some low probability of belonging to the *R. r. ugiensis* population which is consistent with some introgression from *R. r. ugiensis* to *R. r. russata*. However, none of the other IMa2 migration rates between *R. r. russata* and *R. r. ugiensis* or their ancestral populations were significantly different from zero. According to population genetics theory, for populations in which the number of effective gene migrants per generation is less than one, gene flow is too low to counteract other evolutionary processes such as drift (e.g., Wright 1931, Waples and Gaggiotti 2006). Thus, despite gene flow probably being non-zero between these populations, the results suggest that gene flow is generally low and likely insignificant between the northern population *R. r. ugiensis* and the main island population *R. r. russata*.

In contrast, the other satellite population *R. r. kuperi* exhibits substantial gene flow with the main island population. The STRUCTURE analysis groups both island populations together into a single genetic population, suggesting little or no genetic structure between the populations. Also, the mtDNA shows less structure between these populations than between *R. r. ugiensis* and *R. r. russata*: one of the three *R. r. kuperi* haplotypes is shared with *R. r. russata* while one *R. r. russata* haplotype clusters more closely with *R. r. kuperi*, resulting in reduced F_{st} and D_{xy} values. Although reduced

genetic structure is also consistent with incomplete lineage sorting, the results from IMa2, which can differentiate between gene flow and incomplete lineage sorting (Hey 2010), confirm that gene flow is occurring. The number of effective gene migrants per generation from *R. r. russata* to *R. r. kuperi* is 1.15 and statistically significant while the number of migrants from *R. r. kuperi* to *R. r. russata* is even higher at 3.12 per generation, but is not statistically significant. Both estimates of gene flow are thus higher than estimates between *R. r. ugiensis* and *R. r. russata*, and their magnitude suggest that gene flow between *R. r. kuperi* and *R. r. russata* is more substantial and the populations are not as isolated.

R. r. ugiensis is also considerably older than *R. r. kuperi*. The IMa2 model estimates that *R. r. ugiensis* diverged from the main island population 213,000 years ago during the middle Pleistocene, while divergence of the less differentiated *R. r. kuperi* occurred only 15,400 years ago during the very late Pleistocene. These results are consistent with some of the results of the phylogenetic analyses, with both populations diverging later than the estimated date from the *ND2* BEAST tree for the southeastern Solomon clade's most recent common ancestor and the date from *BEAST of the most recent common ancestor of the southeastern Solomon clade and the Guadalcanal subspecies *R. r. rufofronta*. However, *BEAST estimates that the most recent common ancestor of the southeastern Solomon clade occurred only 42,100 years ago, much later than the divergence time estimate from IMa2 for *R. r. ugiensis*. A possible explanation for this discrepancy is that *BEAST estimates of node ages have been shown to be affected by even very low levels of gene flow (Leaché et al. 2014). Thus the gene flow observed in this system is likely affecting the *BEAST results.

Despite the fact that both satellite islands are nearly the same distance from the main island, there are several possible reasons why divergence times might differ. Divergence of both study populations occurred during the middle or late Pleistocene which experienced regular sea level changes (e.g., Bintanja et al. 2005) and the estimated divergence time for *R. r. kuperi* is near the Last Glacial Maximum (26,500–19,000 years ago; Clark et al. 2009), suggesting the possibility that lower sea levels may have facilitated either initial colonization or gene flow that could have caused one or both satellite island populations to merge with the main island population. However, despite the small distances between the main island and satellite islands, channel depths suggest that open water has divided both groups of satellite islands and the main island for the last several hundred thousand years (Diamond and Mayr 1976, Mayr and Diamond 2001, Bintanja et al. 2005). Still, this does not rule out the possibility that the widths of the channels were less, which should facilitate the movement of birds between islands.

A second possible explanation for the young age of *R. r. kuperi* is a past local extinction event. *R. r. kuperi* occurs only on two very small satellite islands (15 and 4.9 km^2; Mayr and Diamond 2001). Small populations are known for their frequent extinctions (e.g., Pimm et al. 1988, Burkey 1995) and thus it is possible that the Rufous Fantail initially colonized those islands around the same time it colonized Ugi. Sometime after colonization, the population became extinct (possibly due to a natural disaster such as a cyclone) and then the Rufous Fantail recolonized the island around 15,000 years ago.

Mode of Divergence

The pattern of morphological divergence closely follows the pattern of neutral genetic structure within the study system, suggesting that the factors that are driving population genetic structure—gene flow and divergence time—may together explain the pattern of morphological divergence. The lack of substantial gene flow between the main island and the strongly morphologically divergent population *R. r. ugiensis* is generally consistent with the prediction of divergence in isolation under the allopatric model. Gene flow estimates for *R. r. ugiensis* are low (< 1 effective gene migrant per generation), demonstrating that extensive morphological divergence in this system has only occurred when gene flow is limited. There is also a negative association between the level of gene flow and the extent of morphological divergence: substantial gene flow is associated with little morphological divergence (*R. r. kuperi*) while limited gene flow is associated with extensive morphological divergence (*R. r. ugiensis*). The additional confounding factor of divergence time prevents these results from directly supporting the prediction that gene flow is inhibiting morphological divergence in this system, which would support the necessity of isolation for divergence and the importance of allopatry. However, none of the results are consistent with the divergence with gene flow model. Thus, this study provides continued support for the prevalence of divergence under the allopatric speciation model in island systems.

Geographic distance, neutral genetic structure, lack of significant gene flow, and the phylogeny of the system support the presence of evolutionarily independent replicate satellite island populations that differ greatly in the degree of morphological divergence, thus providing a unique opportunity to test between models of divergence in an island

system. The finding that extensive morphological divergence has only occurred in the satellite population experiencing limited gene flow is in contrast to several recent studies in support of the divergence with gene flow model that have found morphological divergence between islands in spite of substantial gene flow (Petren et al. 2005, Uy et al. 2009b, Clegg and Phillimore 2010). Several studies comparing populations across archipelagos also generally failed to detect a positive association between the amount of morphological divergence and gene flow-driven genetic structure which indicates that gene flow was not inhibiting morphological divergence (Petren et al. 2005, Clegg and Phillimore 2010). In contrast, this study's results are similar to the pattern documented by Calsbeek and Smith (2003) in which lower morphological divergence occurred between populations experiencing more gene flow. However, this study was unable to control for the confounding factor divergence time and thus was unable to demonstrate that gene flow was most likely inhibiting divergence between islands as Calsbeek and Smith (2003) successfully did.

Divergence Time and Morphology

Divergence times may have also played an important role in determining the pattern of morphological divergence in this system, either together with or instead of gene flow. The fact that *R. r. ugiensis* is nearly 14 times older than *R. r. kuperi* may explain why it has been able to diverge extensively in terms of morphology while *R. r. kuperi* has changed little. For the satellite population *R. r. ugiensis,* the amount of morphological divergence between it and the main island population *R. r. russata* is not unreasonable for the length of time (213,000 years) since divergence. Body size evolution

in particular can be very rapid, including in island systems. Multiple studies have found divergence in body size between populations of birds that have been separated for only a few thousand years (e.g., Clegg et al. 2008, Spurgin et al. 2014), or even diverged in historical times (e.g., Diamond et al. 1989, Clegg et al. 2002, Rasner et al. 2004).

Plumage patterns in birds can also diverge rapidly. A number of other bird systems have been found to have evolved extensive plumage divergence within a few hundred thousand years of population divergence, both in island systems and on continents (e.g., Driskell et al. 2002, Johnson and Cicero 2004, Uy et al. 2009a, Jacobsen and Omland 2012, Warren et al. 2012). Within the Solomon Islands, the Chestnut-bellied Monarch *Monarcha castaneiventris* has similarly evolved high levels of plumage divergence across the archipelago since its most recent common ancestor 478,000–526,000 years ago (Uy et al. 2009a). However, although relatively minor plumage divergence similar to the differences found in *R. r. kuperi* has been shown to evolve in only a few hundred years or less in a few systems (e.g., Yeh 2004, Avery et al. 2014), extensive plumage divergence such as found between *R. r. ugiensis* and the main island may take longer to evolve and is likely very rare between populations that have diverged for only 15,000 years. Of the many bird systems in which molecular dating has been applied, only a few examples are known in which this much plumage divergence has occurred on such a short time scale (Ödeen and Björklund 2003, Milá et al. 2007a, Milá et al. 2007b). Thus it seems plausible that divergence time, possibly together with gene flow, could be limiting plumage divergence in this system.

Selection Pressures on Morphology

Differences in selection pressures or the strength of selection on the two groups of satellite islands could also cause the two populations to diverge to different degrees. Little is known about the selection pressures driving the evolution of body size or plumage patterns in the Rufous Fantail. Evidence that selection has caused divergence in size between islands has been found in several island systems (Clegg et al. 2002, Monceau et al. 2013). While the specific selection pressures are unknown, various factors including niche shape, intraspecific and interspecific competition, predation and parasitism levels, and climate have been hypothesized to drive the evolution of size and shape in island populations (reviewed in Lomolino 2005, Clegg 2010), and could potentially differ across the system.

The most notable change in plumage in this system is the evolution of increased black (melanic) coloration in *R. r. ugiensis*. There are a number of cases of increased melanism in island populations (e.g., Ritchie 1978, Wunderle 1981, King and Lawson 1995, Doucet et al. 2004) including systems similar to this one in which increased melanism is restricted to smaller islands (Uy et al. 2009b, Buades et al. 2013, Uy and Vargas-Castro 2015). Possible reasons why increased melanism might evolve in a population include a genetic association with aggressive behavior, changes in light environment, crypsis, sexual selection, stronger feathers, and thermoregulation (e.g., Hoekstra and Nachman 2003, Horth 2003, Jawor and Breitwisch 2003, Burtt and Ichida 2004, Tanaka 2007, Uy et al. 2009b, Mackinven and Briskie 2014). However, it is not known which, if any, of these selection pressures are influencing the evolution of melanic plumage in this system specifically or why melanism might be selected for in one satellite

island group and not the other. Work by Uy et al. (2009b, unpublished data) has found that increased melanic plumage in Chestnut-bellied Monarch populations on these same two groups of satellite islands is being maintained by selection, implying similar selection pressures across the satellite islands. Because both species occur together, it is possible that the Rufous Fantail may also be experiencing similar selection pressures across the two satellite populations, at least for melanic plumage, in which case selection alone would not explain the difference in plumage divergence patterns of the two satellite island populations.

Conclusion

The presence of evolutionarily independent replicate satellite island populations differing extensively in the amount of morphological divergence provides a unique opportunity to acquire insights into the mode of speciation in island systems. In this system, two demographic factors, gene flow and divergence time, are closely associated with morphological and neutral genetic divergence, and may explain the pattern of divergence across the system. Confounding factors prevented this study from conclusively showing that gene flow is inhibiting divergence in this system, one of the key predictions of the allopatric speciation model. However, extensive morphological divergence in this system is only occurring with low gene flow, providing support for the prevalence of allopatric divergence in island systems.

Table 1. Predicted divergence patterns under the two speciation models.

Speciation Model	Divergence Patterns Consistent with the Model	Comments
Allopatric speciation	Divergent satellite population is isolated, while undifferentiated satellite population exhibits gene flow with main island.	Consistent with both model predictions: divergence occurs only in isolation and gene flow is associated with a lack of divergence.
	Both satellite populations are isolated.	Additional factor needed to explain why one population has not diverged.
Speciation with gene flow	Both satellite populations exhibit gene flow with main island population.	

Table 2. Four measures (mean ± SD) of body size (mm) and mass (g) from individuals in the three study populations. Sample size is in parentheses.

	Wing	Tarsus	Tail	Head-bill	Mass
R. r. kuperi	67.7 ± 4.5 (9)	18.81 ± 0.81 (11)	75.3 ± 3.2 (7)	28.71 ± 0.66 (11)	9.9 ± 0.6 (7)
R. r. russata	65.4 ± 2.6 (11)	18.13 ± 0.86 (11)	72.6 ± 3.0 (12)	28.07 ± 0.69 (11)	9.0 ± 0.6 (11)
R. r. ugiensis	68.1 ± 2.5 (28)	19.18 ± 0.95 (28)	75.4 ± 3.1 (29)	29.30 ± 0.88 (28)	10.1 ± 0.6 (26)

Table 3. Pairwise two sample t-tests (t-statistic [p-value]) comparing body size measurements and mass among the three populations. Values in bold are significant after Holm-Bonferroni correction.

	Wing	Tarsus	Tail	Head-bill	Mass
R. r. kuperi vs.	1.35	1.89	1.82	2.24	2.99
R. r .russata	(0.20)	(0.07)	(0.09)	(0.04)	(0.01)
R. r. kuperi vs	-0.29	-1.23	-0.12	-2.27	-1.14
R. r. ugiensis	(0.78)	(0.23)	(0.91)	(0.03)	(0.28)
R. r. russata vs	-2.98	**-3.32**	-2.76	**-4.67**	**-5.17**
R. r. ugiensis	(0.008)	**(0.003)**	(0.01)	**(< 0.001)**	**(< 0.001)**

Table 4. Results of principal component analysis on size measurements between two of the study populations, *R. r. russata* (n = 11) and *R. r. ugiensis* (n = 26). The loadings on the first two principal components, the proportion of variance explained by each, and the results of a two sample t-test (t-statistic [p-value]) comparing the principal components between the populations are given.

	PC1	PC2
Loadings		
Wing	0.48	0.45
Tarsus	0.39	-0.49
Tail	0.45	0.54
Head-bill	0.46	-0.51
Mass	0.45	-0.08
Proportion of variance explained	0.49	0.20
Two-sample t-test	-6.35 (< 0.001)	0.96 (0.35)

Table 5. Summary of genetic sequence data for the three Rufous Fantail study populations used in this study. bp = number of base pairs sequenced, Hap. = number of haplotypes, π = nucleotide diversity

					R. r. kuperi		*R. r. russata*		*R. r. ugiensis*	
Marker	bp	SNP sites	Samples Sequenced	Phase probability > 0.8	Hap.	π	Hap.	π	Hap.	π
mtDNA										
ND2	810	30	58 (100%)		3	0.0024	12	0.0054	2	0.0017
Nuclear										
15246	410	3	55 (95%)	55 (100%)	3	0.0014	4	0.0014	3	0.0021
BFIB	438	5	58 (100%)	57 (98%)	1	0.0000	3	0.0009	3	0.0043
MYO	511	4	58 (100%)	58 (100%)	4	0.0013	4	0.0007	3	0.0002
RHO	490	4	58 (100%)	58 (100%)	4	0.0009	2	0.0003	3	0.0006
TGFB2	439	11	58 (100%)	55 (95%)	6	0.0083	11	0.0063	4	0.0063

Table 6. F_{st} and D_{xy} estimates of population structure among the three study populations. Bold numbers indicate significant F_{st} values after Holm-Bonferroni correction.

	F_{st}			D_{xy}		
Marker	*R. r. kuperi* vs *R. r. russata*	*R. r. kuperi* vs *R. r. ugiensis*	*R. r. russata* vs *R. r. ugiensis*	*R. r. kuperi* vs *R. r. russata*	*R. r. kuperi* vs *R. r. ugiensis*	*R. r. russata* vs *R. r. ugiensis*
mtDNA						
ND2	**0.151**	**0.676**	**0.526**	0.006	0.0104	0.0125
Nuclear						
15246	0.042	0.144	0.039	0.001	0.0021	0.0017
BFIB	-0.001	**0.225**	**0.198**	0.000	0.0034	0.0036
MYO	0.103	**0.283**	0.049	0.001	0.0009	0.0004
RHO	0.034	0.015	-0.005	0.001	0.0008	0.0005
TGFB2	**0.095**	0.089	0.053	0.008	0.0074	0.0076
Mean nuclear	*0.055*	*0.151*	*0.067*	*0.002*	*0.0029*	*0.0027*

Figure 1. Rufous Fantail *Rhipidura rufifrons* study system in southeastern Solomon Islands. Colors indicate ranges of the three subspecies. Illustrations of Rufous Fantails reproduced from Dutson 2011.

Figure 2. Values of individual birds for the first two principal components (PC1 and PC2) from the principal component analysis of morphometric measurements showing the division between the populations along the PC1 axis. Filled circles represent *R. r. ugiensis* individuals and open circles represent *R. r. russata* individuals.

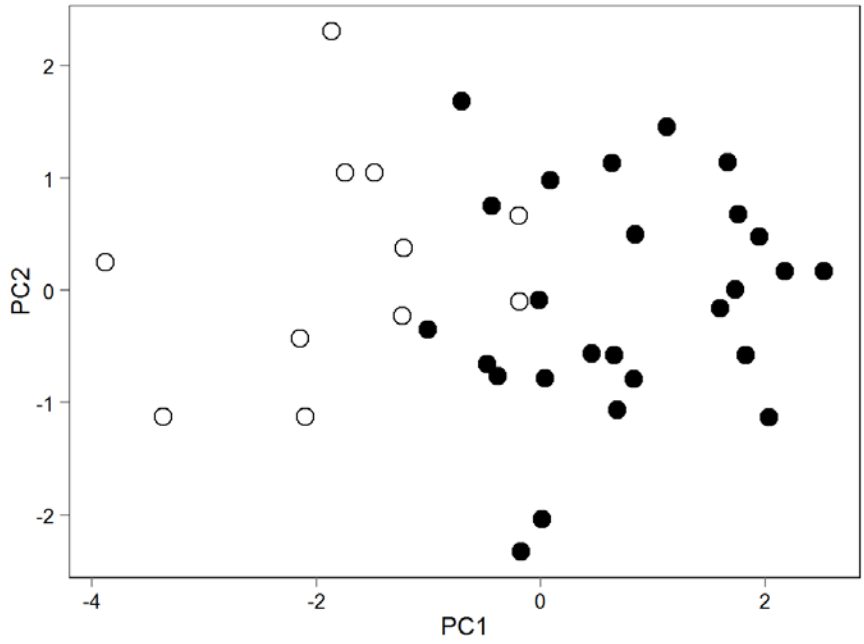


Figure 3. Measurements of two plumage patch sizes, extent of white on throat and extent of black in the central rectrices, from the three study populations. Different letters indicate significant differences between populations. Illustrations of Rufous Fantails reproduced from Dutson 2011.

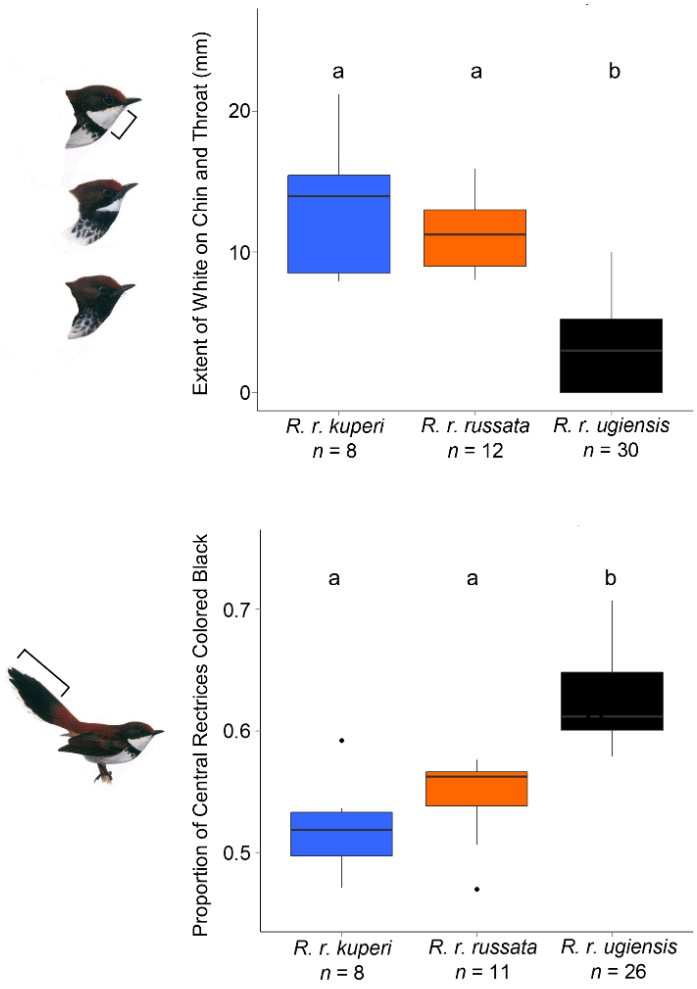


Figure 4. Haplotype network of mtDNA *ND2* sequences from the three study populations. The abundance of each haplotype is proportional to the area of the corresponding circle.

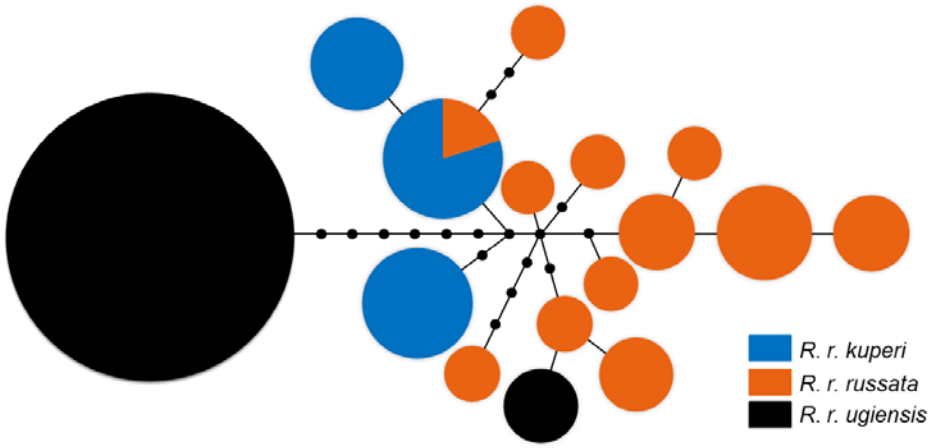


Figure 5. STRUCTURE plot showing composition of genetic populations based on the most likely number of populations ($K = 2$) using the five nuclear markers.

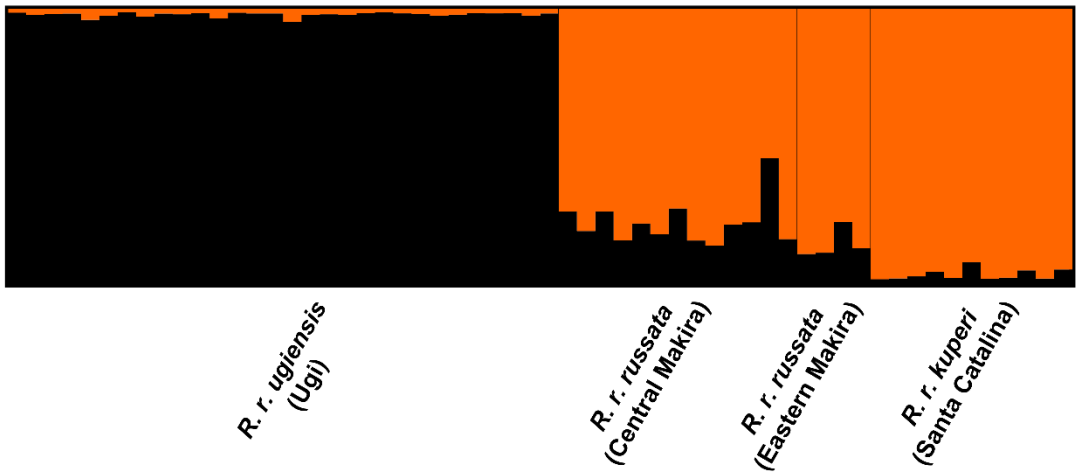


Figure 6. Bayesian BEAST phylogenetic tree showing relationships among *ND2* haplotypes of Solomon Islands Rufous Fantail populations and two outgroups, *R. dryas* and *R. teysmanni*. Numbers at nodes indicate posterior probabilities. Colors indicate different subspecies of the Rufous Fantail in the Solomon Islands.

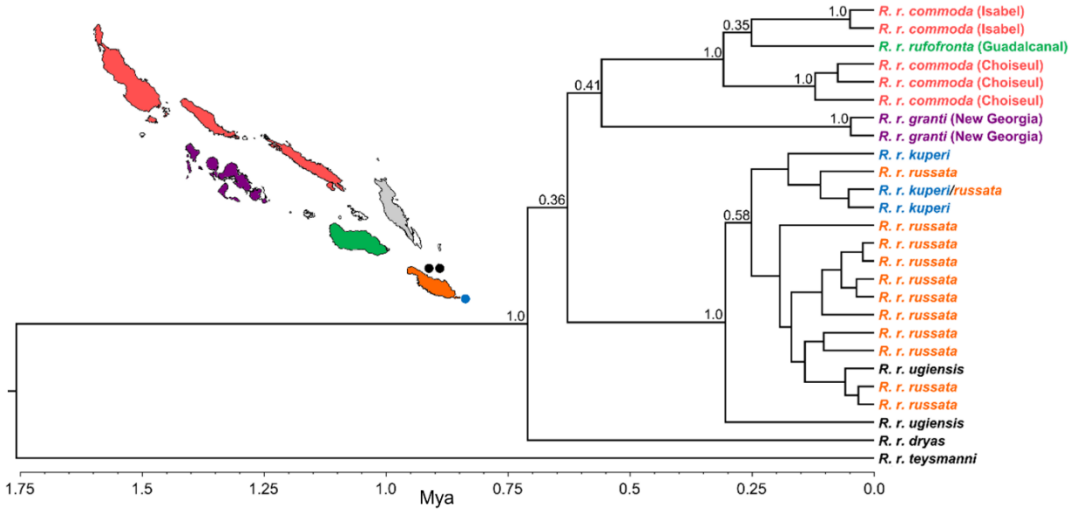


Figure 7. Bayesian *BEAST phylogenetic trees showing relationships among study taxa and two outgroups, *R. r. rufofronta* and *Chaetorhynchus papuensis*, using mtDNA and nuclear markers combined (top) and nuclear markers alone (bottom). Posterior probability values are presented at the nodes while the bars show the 95% HPD node age intervals.

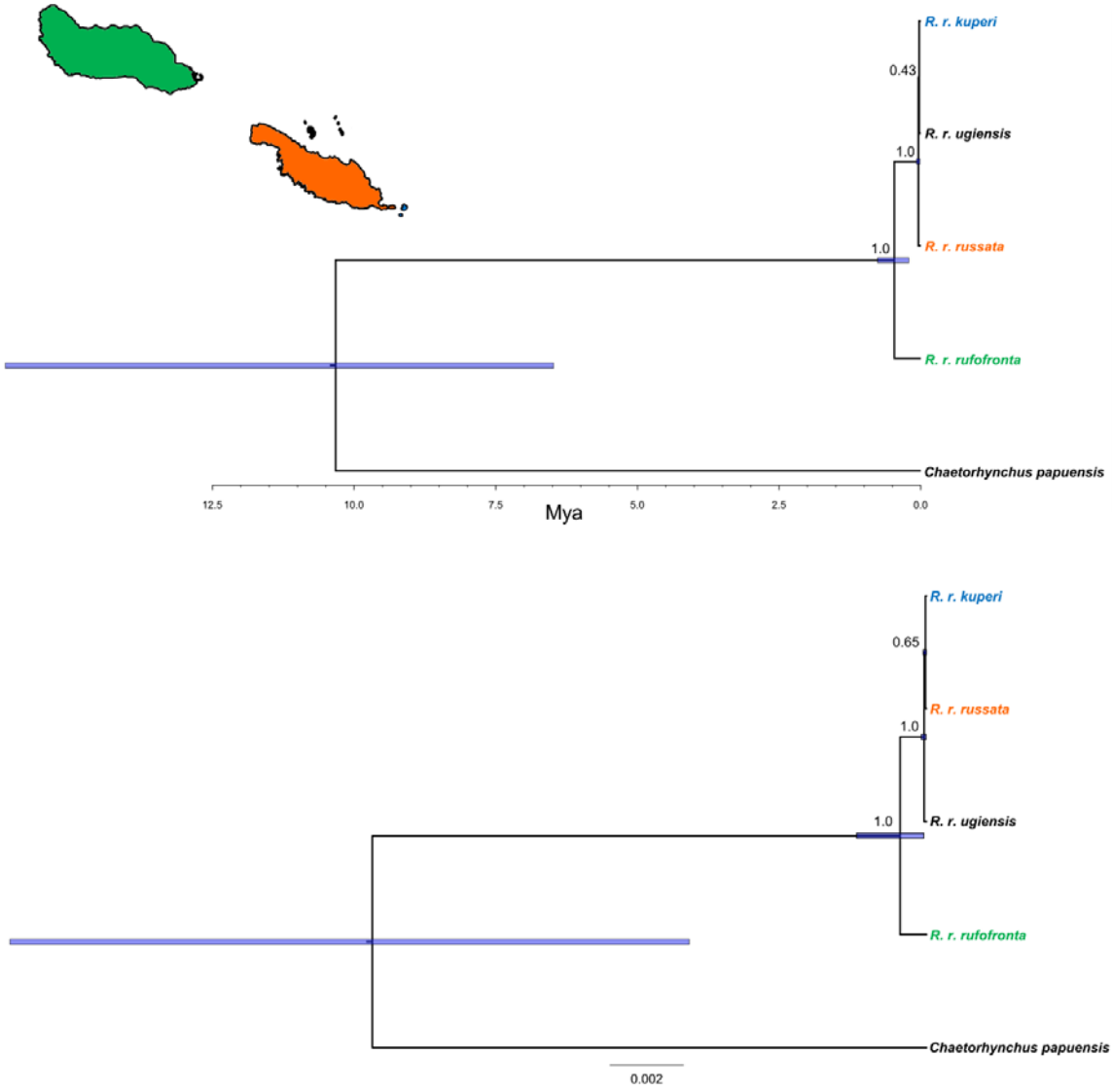


Figure 8. Posterior probability distributions of IMa2 parameter estimates: (a-b) population size parameter θ, (c-f) migration rates, and (g) divergence times.

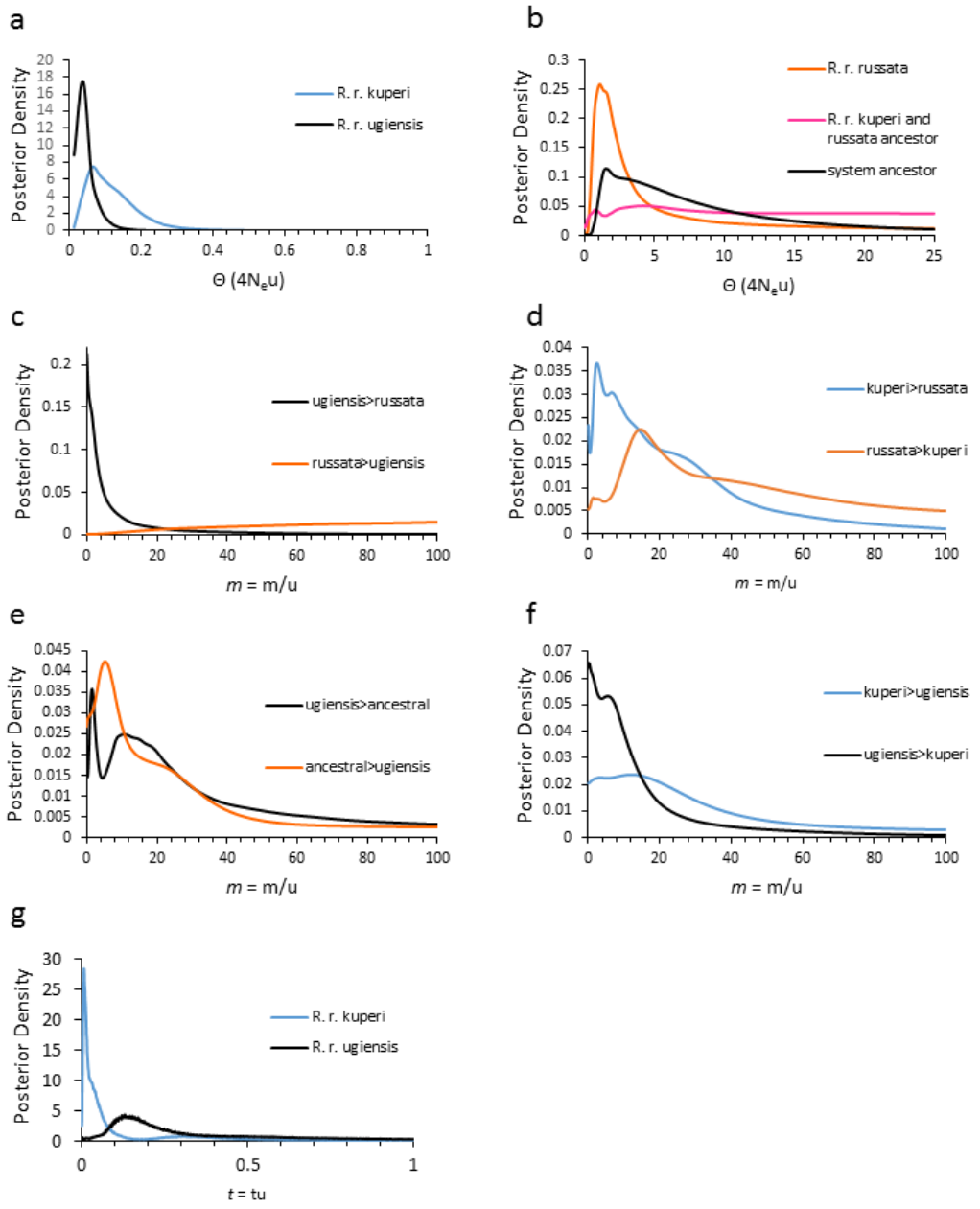
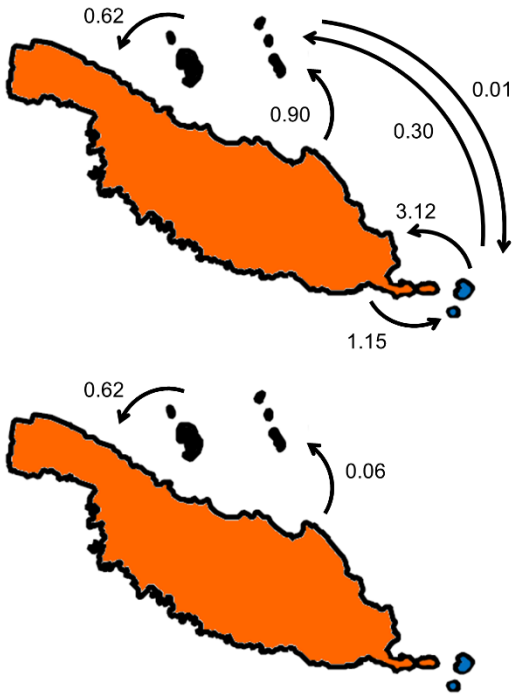


Figure 9. Estimates of the number of effective gene migrants per generation ($2N_em$) from IMa2 between (top) the three study populations and (bottom) *R. r. ugiensis* and the ancestor of *R. r. kuperi* and *R. r. russata*.



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