

# PLUMAGE AND SONG DIFFERENCES MEDIATE SPECIES RECOGNITION BETWEEN INCIPIENT FLYCATCHER SPECIES OF THE SOLOMON ISLANDS

J. Albert C. Uy,<sup>1,2</sup> Robert G. Moyle,<sup>3</sup> and Christopher E. Filardi<sup>4</sup>

<sup>1</sup>Department of Biology, Syracuse University, Syracuse, New York 13244

<sup>2</sup>E-mail: jauy@syr.edu

<sup>3</sup>Natural History Museum and Biodiversity Research Center, and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045

<sup>4</sup>Center for Biodiversity and Conservation, American Museum of Natural History, New York 10024

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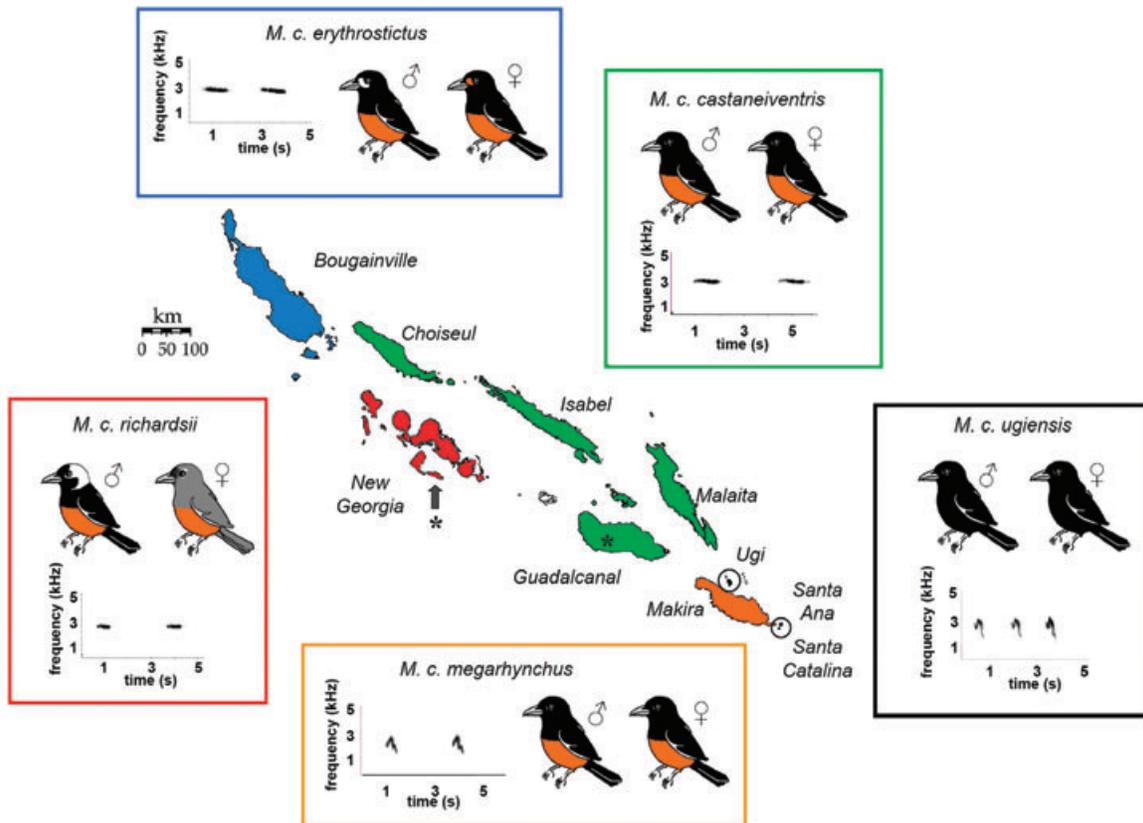
Changes in mating signals among populations contribute to species formation. Often these signals involve a suite of display traits of different sensory modalities ("multimodal signals"); however, few studies have tested the consequences of multimodal signal divergence with most focusing on only a single divergent signal or suite of signals of the same sensory modality. Populations of the chestnut-bellied flycatcher *Monarcha castaneiventris* vary in song and plumage color across the Solomon Islands. Using taxidermic mount presentation and song playback experiments, we tested for the relative roles of divergent song and color in homotypic ("same type") recognition between one pair of recently diverged sister taxa (the nominate chestnut-bellied *M. c. castaneiventris* and the white-capped *M. c. richardsii* forms). We found that both plumage and song type influenced the intensity of aggressive response by territory-owners, with plumage color playing a stronger role. These results indicate that differences in plumage and song are used in homotypic recognition, suggesting the importance of multimodal signal divergence in the evolution of premating reproductive isolation.

**KEY WORDS:** *Monarcha*, multimodal signals, premating reproductive isolation, species recognition, speciation.

Animals often use a suite of signals of different sensory modalities in conspecific interactions ("multimodal signals," Rowe 1999; Candolin 2003; Partan and Marler 2005). For instance, many bird species use complex songs and colorful plumage in competing for or attracting mates (Andersson 1994). These multimodal signals are shaped by sexual selection and are often very distinct among closely related species, inspiring the hypothesis that divergent sexual selection is important in the speciation process (Darwin 1871; West-Eberhard 1983; Price 2007). However, most studies that link sexual selection and speciation do not explicitly test the role of multimodal signals in premating reproductive isolation or species recognition, often focusing on only a single divergent signal or a

suite of signals of the same sensory modality (e.g., Boake et al. 1997; Seehausen and van Alphen 1998; Gray and Cade 2000; Uy and Borgia 2000; Boughman 2001; Irwin et al. 2001; Masta and Maddison 2002; Seddon and Tobias 2007). Because many species use a unique set of multimodal signals in conspecific interactions (Rowe 1999; Candolin 2003), it is important to determine the role of divergent multimodal signals in species recognition and the evolution of reproductive isolation (e.g., Baker and Baker 1990; Patten et al. 2004; Mullen et al. 2007).

Populations of the chestnut-bellied flycatcher *Monarcha castaneiventris* show striking variation in plumage color and song structure throughout the Solomon Islands (Fig. 1). This striking



**Figure 1.** Distribution, and song and color variation among populations of the *Monarcha castaneiventris* complex of the Solomon Islands. These taxa are variably classified as subspecies, allopecies, or full species (Mayr 1942; Mayr and Diamond 2001; Filardi and Smith 2005), but, for simplicity, we follow Mayr's (1942) original taxonomy, which classifies the forms as subspecies of the variable *M. castaneiventris* complex. Divergent subspecies classified by their plumage color and overall morphometrics are color-coded: nominate chestnut-bellied form (*M. c. castaneiventris*) in green, Makira Island chestnut-bellied form (*M. c. megarhynchus*) in orange, all-black form (*M. c. ugiensis*) in black (islands circled on map), white-capped form (*M. c. richardsii*) in red, and Bougainville form (*M. c. erythrostrictus*) in blue. Populations in which mount presentation and song playback experiments were conducted are marked with asterisks.

differentiation represents the early stages of species formation (Filardi and Smith 2005), and it featured prominently in the development of Mayr's (1942) early assertion that geographic isolation is key to the speciation process. As such, this radiation provides us with a unique natural opportunity to understand the role of multimodal signal divergence in speciation.

Mayr (1942) discussed several major *M. castaneiventris* color forms endemic to the Solomon Islands that vary in body size and, most dramatically, in plumage color, sexual dimorphism, and song structure (Fig. 1). The color variation ranges from an entirely iridescent blue-black form to a chestnut-bellied and white-capped form, and sexual dimorphism ranges from being monochromatic to extremely dichromatic (Fig. 1). Variation in song structure ranges from a simple descending whistle note to a more complex upslurred then down-slurred whistle note (Fig. 1). Three other chestnut-bellied *Monarcha* species appear to form a natural grouping with *M. castaneiventris* but do not inhabit any of the major islands in the Solomon Archipelago (Mayr and Diamond

2001). These include the black-faced Monarch *M. melanopsis* of Australia, the black-winged Monarch *M. frater* of New Guinea, and the islet Monarch *M. cinerascens*, which is found exclusively on islets throughout parts of the Solomon Islands and to the west through eastern Indonesia. All three are monochromatic, and have chestnut bellies and gray upper parts with *M. melanopsis* having a small black mask, and *M. frater* having a larger black mask, and black remiges and tail. The most recent treatment of the *M. castaneiventris* complex classifies some insular populations as allopecies (i.e., *M. c. richardsii* and *M. c. erythrostrictus*), and groups the entire Solomon endemic clade with the three gray Australasian flycatchers to form the larger *M. melanopsis* superspecies complex (Mayr and Diamond 2001; but see Filardi and Smith 2005). For simplicity, however, we follow Mayr's (1942) original taxonomy, which classifies the Solomon endemics as subspecies of the variable *M. castaneiventris* complex.

In this study, we derived a molecular phylogeny based on nuclear and mitochondrial genes for the entire *M. melanopsis*

superspecies complex, which allowed us to infer the evolutionary history of this group and explore the appropriate taxonomic classification of this variable complex. Using this phylogeny, we then chose two extremely variable sister taxa, and experimentally tested for the relative roles of divergent song and plumage color in species recognition and premating reproductive isolation. Mating trials would provide a more direct assay of premating isolation; however, such experiments are logistically difficult to conduct in wild populations and a remote region like the Solomon Islands. Instead, we conducted taxidermic mount presentation and song playback experiments to determine if territory-owners use divergent song and plumage in recognizing sexual competitors, which is widely used to infer premating isolation between taxa (e.g., Irwin et al. 2001; Grant and Grant 2002a,b; Patten et al. 2004; Balakrishnan and Sorenson 2006; Seddon and Tobias 2007). The logic behind this test is that if females use taxon-specific plumage and song in choosing among potential mates, selection should favor males that likewise discriminate among potential competitors based on plumage and song. Discriminate responses by territorial birds to one or the other signal type therefore suggests that females use the same signals in mate choice, and hence the presence of premating barriers between taxa. Experiments in lazuli and indigo buntings (*Passerina* spp.; Baker and Baker 1990; Baker 1991), and song sparrows (*Melospiza melodia* ssp.; Searcy et al. 1997; Patten et al. 2004) confirm that traits used by males in recognizing sexual competitors/conspecifics are also used by females in mate choice. Similarly, in túngara frogs *Physalaemus pustulosus*, males and females differ in their overall responsiveness to heterospecific and conspecific songs but both sexes respond more strongly to conspecific songs and heterospecific songs of more closely related species than heterospecific songs of distantly related taxa (Bernal et al. 2007). Hence, with the assumption that traits used in conspecific or homotypic recognition are also used in mate choice, we use mount presentation and song playback experiments to test if divergent signals are used in homotypic (“same type”) recognition and infer premating reproductive isolation between the two *M. castaneiventris* color forms.

## Methods

### NATURAL HISTORY OF STUDY TAXON

Information on basic natural history of *Monarcha castaneiventris* is based primarily on long-term observations of *M. c. richardsii* (Filardi 2003; Filardi and Smith 2008), and preliminary observations of the other subspecies during collecting and mist netting. *Monarcha* flycatchers are insectivorous, leaf-gleaners found primarily in the middle and lower strata of forests. They are socially monogamous and breeding has been recorded throughout the year. Incidental evidence, however, suggest that individual birds do not raise more than a single clutch during an annual cycle (Filardi

2003; C. E. Filardi, unpubl. data). Pairs defend breeding or nesting territories from other breeding pairs, and are most aggressive prior to and during an active breeding attempt (C. E. Filardi, unpubl. data). Song playback experiments indicate that territory-owners from all subspecies respond aggressively to homotypic songs (J.A.C. Uy and C. E. Filardi, unpubl. data). In *M. c. richardsii*, adult males are especially aggressive and are easily caught with song playback. Females, however, have also been caught using this technique, suggesting that both sexes defend territories. In *M. c. erythrostictus* females are attracted to playbacks but never approach the speakers, unlike males who are extremely aggressive and readily attack speakers. For the monochromatic forms (*M. c. castaneiventris*, *M. c. megarhynchus*, and *M. c. ugiensis*) determining sex during observations in the field is not possible; however, we have caught multiple individuals with song playbacks within a single (presumed) territory, suggesting that both sexes defend territories. Even in the taxa in which both sexes aggressively respond to playbacks, males are clearly more aggressive and are typically caught first (as confirmed by the presence of testes during mount preparations). In addition, mount presentation experiments in the dichromatic white-capped form indicate that males preferentially attack mounts of adult males and solicit matings from mounts of females, indicating that plumage color is used in sexual interactions and likely in mate choice (Filardi and Smith 2008).

### TAXON SAMPLING

We augmented the taxon sampling in Filardi and Smith (2005) by sampling the remaining major taxa or populations to complete the *M. melanopsis* superspecies complex (Supporting Table S1). In total, we sequenced and analyzed 39 individuals representing 10 island groups spanning the Solomon Archipelago. In addition, we included seven birds from the three other allospecies of the *M. melanopsis* superspecies complex (*M. melanopsis*, *M. frater* and *M. cinerascens*) in our analyses. The Rennell Shrikebill *Clytorhynchus hamlini* is closely related to the *M. melanopsis* superspecies complex (Filardi and Moyle 2005; Filardi and Smith 2005); hence, we used the shrikebill as the outgroup for our analyses. We sequenced 1037 bp of the mitochondrial nicotinamide adenine dinucleotide dehydrogenase (*ND2*), and ca. 649 bp of the 3' end of the noncoding Control Region subunit 2 (*DII*) following established protocol for *Monarcha* flycatchers (Filardi and Smith 2005). We also sequenced the nuclear intron *TGF $\beta$ 2* using primers and protocol described in Primmer et al. (2002) and Carling and Brumfield (2008).

### PHYLOGENETIC ANALYSES

Phylogenetic hypotheses were reconstructed using Bayesian inference (BI) and maximum likelihood (ML). ML searches were conducted in PAUP\* 4.0b10 (Swofford 2002) using TBR branch

swapping and 100 random taxon additions. The model of nucleotide substitution and parameter estimates were obtained with Modeltest 3.7 (Posada and Crandall 1998) under the AIC criterion. Support for nodes in the ML phylogeny was obtained by analysis of 100 nonparametric bootstrap replicates of the dataset. Bayesian analyses used MrBayes 3.1.2 (Ronquist and Huelsenbeck 2001) and incorporated different models of nucleotide substitution for each of five data partitions (three codon positions, *DIII*, and *TGF $\beta$ 2*), determined with MrModeltest 2.2 (Nylander 2004). Two independent runs of 10 million generations, subsampled every 1000 generations, were used to obtain samples from the posterior distribution of trees and parameter estimates. The BI consensus tree was converted to a chronogram with penalized likelihood using the program *r8s* (Sanderson 2003).

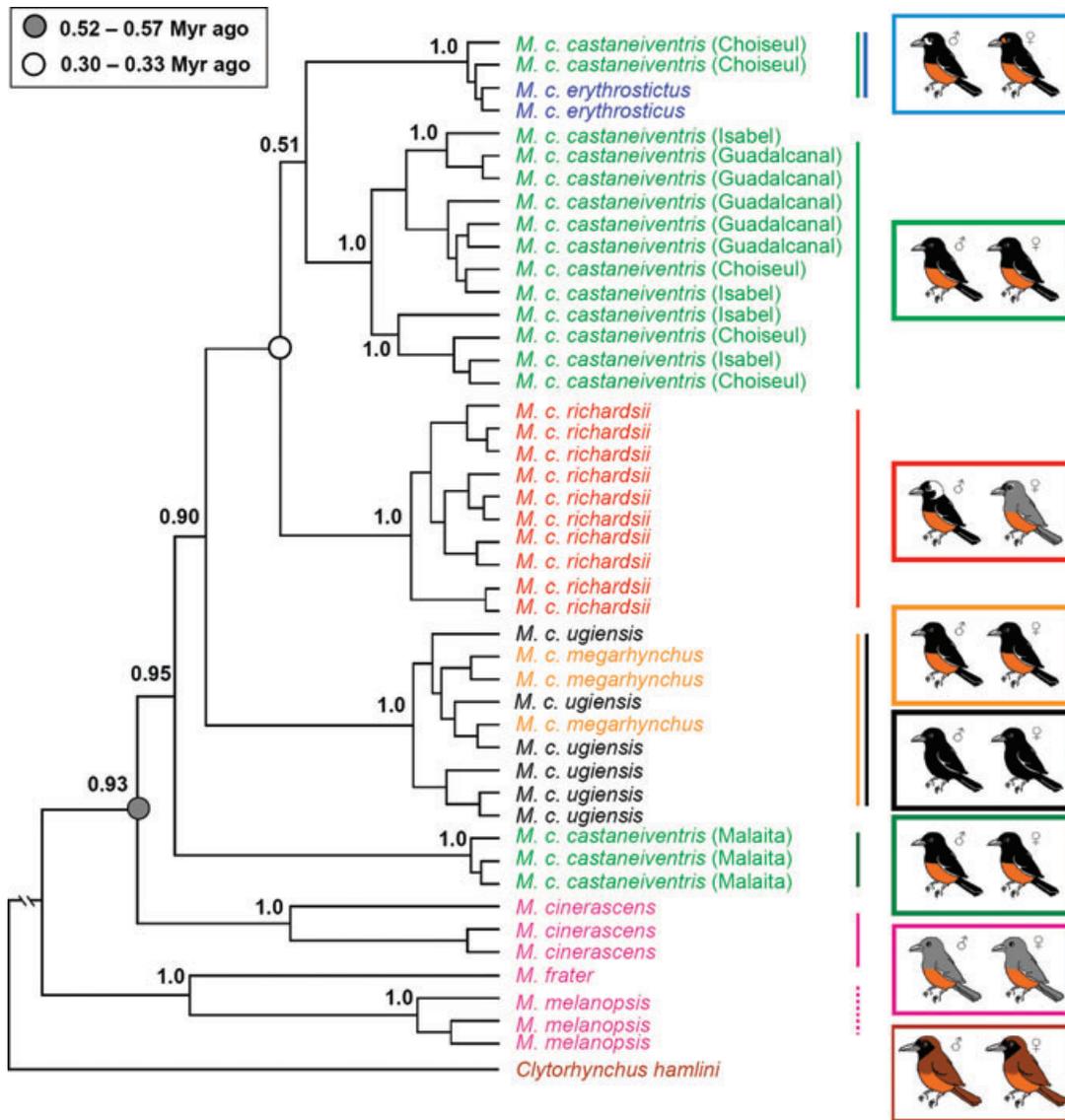
The substitution rate of 2% per million years for mitochondrial genes is widely used to calibrate avian phylogenies; however, this rate was derived only for the cytochrome *b* gene and there is considerable debate on the validity and utility of using this rate across distantly related avian taxa and specific mitochondrial genes (e.g., García-Moreno 2004; Lovette 2004). The use of fossil record and geological calibrations provide more robust and realistic estimations of rates of divergence, but unfortunately these data are not available for *Monarcha* flycatchers. A recent study, however, derived a divergence rate for *ND2* that is more appropriate and useful for our study. Arbogast et al. (2006) calculated the divergence rate for *ND2* in other passerine birds and used island ages as calibration points. The use of island ages provides reliable maximum age estimates and the use of island Passerines presents similar evolutionary circumstances to those that produced our dataset, including small effective population sizes and potential founder effects (Filardi and Moyle 2005). Therefore, we use this *ND2*-specific rate of 0.020 to 0.022 substitutions per site per lineage million years to obtain a rough estimate of divergence times within the group (Arbogast et al. 2006). These ages differ slightly from those derived for the same nodes by Filardi and Moyle (2005). Some discrepancy may be due to differences in taxon sampling, but the majority can likely be traced to a different rate calibration. Filardi and Moyle (2005) used the *ND2* rate from Drovetski et al. (2004), which was based on unpublished data. The published rate based on those data (Arbogast et al. 2006) was refined downward. Before using this single rate across the entire phylogeny, we tested the *ND2* data for clock-like evolution using a likelihood-ratio (LR) test. Likelihood scores from ML searches, with and without the molecular clock enforced, were determined in PAUP. We assessed significance by comparing two times the difference in log likelihoods to a  $\chi^2$  distribution with  $n - 2$  degrees of freedom, where  $n$  was the number of taxa.

## EXPERIMENTAL TEST OF CONSPECIFIC RECOGNITION

Because the distinct color forms are primarily allopatric throughout their range (Fig. 1), it remains unclear if the observed multimodal signal divergence would result in premating reproductive isolation if individuals of different color forms establish secondary contact. We can, however, simulate secondary contact and experimentally infer the presence of reproductive isolation by presenting territory-owners with varying taxidermic mounts and song recordings, and assaying their behavioral response to these stimuli (e.g., Irwin et al. 2001; Grant and Grant 2002a,b). The use of mount presentation and song playback experiments as an alternative test to mating trials is widely used in the avian literature (Baker 1991; Irwin et al. 2001; Grant and Grant 2002a,b; Patten et al. 2004), especially if the experiment incorporates appropriate controls (e.g., ecologically similar, sympatric heterospecific stimuli).

Based on our molecular phylogeny, we chose and tested a sister taxa pair that show the most distinct differentiation in plumage color and extent of sexual dichromatism: the monochromatic chestnut-bellied *M. c. castaneiventris* and the dichromatic white-capped *M. c. richardsii* forms (Fig. 2). Because *Monarcha* flycatchers defend territories by calling, we searched for territory-owners by walking along trails and finding calling pairs. At each territory, we then randomly chose one of five treatment groups: (1) homotypic mount and song, (2) homotypic mount with heterotypic *M. castaneiventris* ssp. song, (3) heterotypic *M. castaneiventris* ssp. mount with homotypic song, (4) heterotypic *M. castaneiventris* ssp. mount and song, and (5) heterospecific mount and song (see Fig. 3). For the heterospecific control, we chose a sympatric and distantly related species that share a similar ecological niche to *M. castaneiventris* ssp., the golden whistler *Pachycephala pectoralis* ssp. We took the global positioning system (GPS) coordinates of each territory to ensure that we did not return to the same pair for subsequent experiments.

Kroodsma et al. (2001; see also Kroodsma 1989) advocate an experimental protocol that uses a unique stimulus for each trial to avoid “simple pseudoreplication,” which is a design that uses a single exemplar to represent an entire class of signals. Using a new exemplar for each mount presentation trial is not feasible; however, we avoided “simple pseudoreplication” by using multiple exemplars per taxon, and a mixed model, nested analysis of variance (ANOVA) for hypothesis testing (details below). Two adult males were caught and prepared for taxidermic mounts for each taxon (i.e., *M. c. richardsii* and *P. p. melanoptera* from Tetepare Island, and *M. c. castaneiventris* and *P. p. cinnamomea* from Guadalcanal Island). The use of additional mounts may provide a better representation of the two plumage types; however, variation in plumage color between the two *Monarcha* forms is qualitative (i.e., presence or absence of white cap) and so the limited number of taxidermic mounts used in the experiments



**Figure 2.** Chronogram (time-calibrated phylogeny) of the *Monarcha melanopsis* superspecies complex derived from Bayesian phylogenetic inference using penalized likelihood. For the Solomon endemics, we use the same color scheme as in Fig. 1. The three species that are not endemic to the Solomon Islands (*M. cinerascens*, *M. frater*, *M. melanopsis*) but are part of the superspecies complex are in purple. Based on a more general *Monarcha* phylogeny that involved 14 species distributed throughout Australasia and the South Pacific (Filardi and Smith 2005), we used the Rennell Shrikebill *Clytorhynchus hamlini* (brown) as the outgroup. For our analyses, we used the following model of nucleotide substitution: ND2 1st codon positions=HKY+I, ND2 2nd codon positions=HKY, ND2 3rd codon positions=GTR+I, DII=GTR+I+G, TGF=GTR. Nodal support based on Bayesian probability scores is provided above each branch. Node ages are based on estimates from ND2-specific calibration (see Methods).

effectively represent each form. For song playbacks, we recorded long-range advertisement songs (“whistles”) from six different individuals for each taxon with a Marantz (Mahwah, NJ) PMD670 digital recorder set at 16-bit PCM, 48 kHz sampling rate, and fitted with a Sennheiser (Old Lyme, CT) shotgun microphone. Comparison of whistle notes between the two taxa indicate that they differed primarily in the length of the whistle note, and that the six recordings per taxon we used for our experiments effectively represent each song type (Supporting Fig. S1). The

choice of mount and recording used for each trial was chosen randomly.

In addition to whistles, two more call types are used by *M. castaneiventris*: a raspy call used during aggressive interactions and a soft chatter used during interactions in larger aggregations or courtship between sexes (Filardi and Smith 2008). The raspy and chatter call types are structurally similar among all color forms, and so we only used whistles in our playback experiments. Mounts were perched on a locally collected sapling ca. 2-m tall

and placed adjacent to vegetation suitable for perching by territorial birds. Beneath the mount's perch, we fitted a small speaker (Mini-Amplifier; RadioShack Corp., Fort Worth, TX) and a digital player (Ipod Shuffle; Apple, Inc., Seattle, WA), concealed by leaves collected from the habitat. After set up, the digital player played 3 min of silence before broadcasting whistles to start the experiment. Based on preliminary observations, territory-owners attacked homotypic mounts within 2 min; hence, each trial lasted for 3.5 min (210 sec). Observations were conducted ca. 15–20 m away from the mount by two observers who were concealed in thick vegetation. To ensure consistency in behavioral observations, all experiments were ran by a single observer (JACU), aided by a local field guide who helped in spotting birds in the canopy or before approach. Observations were spoken into a recorder (Marantz PMD670 digital recorder and built-in microphone), which allowed for an accurate quantification of behavior by a single observer and the recording of territorial birds responding to our mounts and song playbacks. All trials were ran between 0630 to 1100 h and 1500 to 1730 h, the time periods in which individuals were observed to sing most often from territories.

Behavioral responses were noted throughout the experiment, and we focused our analyses on behaviors that were likely assays of aggression as well as conspecific recognition: (1) number of attacks or pecks at the mount, (2) time spent perched on the mount's stick, (3) time spent perched on adjacent vegetation, (4) number of flights near the mount (< 2 m) without contact, (5) time spent within 2 m of the mount emitting raspy, aggressive calls, (6) time spent > 2 m from the mount emitting raspy calls, (7) time spent < 2 m of the mount emitting whistles, (8) time spent > 2 m from the mount emitting whistles, (9) time spent < 2 m of the mount emitting chatter calls, (10) time spent > 2 m from the mount emitting chatter calls, (11) time spent calling in the canopy, and (12) total time spent in the canopy away from the mount. We used the 2-m stick on which the mount was perched as a reference for our measure of distance from the mount. These behavioral variables are likely correlated; however, each may provide unique insights into the behavioral responses of territory-owners. For instance, number of attacks, time spent in adjacent vegetation, and number of flights near the mount are all likely assays of aggression (e.g., Pearson and Rohwer 2000). However, attacks or pecks at the mount are clearly aggressive, whereas flights near the mount or time spent in adjacent vegetation by territory-owners may also represent inspection behavior. Likewise, because three call types are used by *M. castaneiventris* for different social interactions (e.g., advertisement, aggression, courtship; Filardi and Smith 2008), we did not simply assay time spent calling but categorized the responses (recorded during the experiments) based on the three call types and the relative distance in which they responded with these vocalizations. Time spent in the canopy and calling > 2 m from

the mount are also distinct behaviors, as a territory-owner staying in the canopy suggests lack of recognition whereas a territory-owner approaching the mount suggests some form of recognition or inspection. Hence, we believe that these behavioral categories, though correlated, may provide important insights, and we used a principal component analysis (PCA) to collapse them into fewer orthogonal scores that characterized overall aggression or recognition (Filardi and Smith 2008). We used the varimax with Kaiser normalization method to extract principal component (PC) scores, with only PC scores with an eigenvalue of > 1 allowed to enter the model. To interpret the PC scores, we considered the behavioral factors with loadings of > 0.50 or < -0.50 (see Supporting Table S2).

Because territory-owners typically ignored the golden whistler stimuli (Fig. 2), we ran two separate analyses to ensure that our results were not driven by the inclusion of the golden whistler trials. First, we ran a mixed-model, nested ANOVA that excluded the golden whistler trials, testing for the effects of plumage and song type (fixed factors) with specific mount and song recording (random factors) nested within the plumage and song type, respectively. This is the experimental and statistical design advocated by Kroodsma et al. (2001; see also Kroodsma 1989) and is used effectively by others in playback experiments with limited number of exemplars (Grant and Grant 2002a,b; Newman et al. 2006). Second, we ran a mixed model ANOVA that included the golden whistler trials. However, because the inclusion of the golden whistler trials resulted in an unbalanced experimental design (e.g., no combination of homotypic *M. castaneiventris* plumage and golden whistler song treatment, etc.), we could not run a similar nested ANOVA, and instead used the five treatment groups as the independent factor and PC score as the dependent variable. For each trial, a unique combination of mount and song stimuli was used (e.g., 12 combinations from the two mounts, six songs per taxon); hence, simple pseudoreplication was not an issue. To explore the patterns among treatment groups, we then ran pairwise post hoc tests (Fisher's protected least square differences, as in Grant and Grant 2002a), and a linear contrast for the main effect of the five treatment groups. The linear contrast was constructed using the following coefficients (1, 0.5, 0, -0.5, -1) that corresponded to the five treatment groups in the following order (homotypic mount and song, homotypic mount and heterotypic *M. castaneiventris* song, heterotypic mount and homotypic song, heterotypic mount and song, and golden whistler mount and song). A significant linear relationship, therefore, indicates that the intensity of response declines linearly. Because our dataset may not meet the assumptions of parametric tests, we also used randomization tests to calculate probability values for hypotheses testing for all analyses (i.e., compared the *F* statistics to a randomized distribution of our dataset; Cassell 2002). We present the results from randomization and standard parametric

**Table 1.** Mixed-model, nested ANOVA of aggressive response (PC1) by territory-owners to taxidermic mount presentations and song playbacks, excluding the golden whistler (heterospecific control) trials. Probability values (*P*) from parametric ANOVA and randomization tests (in parenthesis) are provided (model  $R^2 = 0.335$ ).

Factor	df	Type III Sum of Squares	<i>F</i>	<i>P</i>
Taxon	1, 79	0.013	0.01	0.906 (0.890)
Plumage type	1, 1.71 <sup>1</sup>	22.78	247.00	0.007 (0.008)
Mount (plumage)	2, 79	0.20	0.11	0.899 (0.906)
Song type	1, 10.08 <sup>1</sup>	3.96	7.58	0.020 (0.020)
Recording (song)	10, 79	5.21	0.56	0.839 (0.850)
Plumage × Song	1, 79	4.60	4.97	0.029 (0.018)
Residuals	79	73.06		

<sup>1</sup>Satterthwaite corrected degrees of freedom.

tests in Table 1. Statistical analyses were performed using SAS version 8.1 (SAS Inst., Inc., Cary, NC.).

For the chestnut-bellied *M. c. castaneiventris*, we tested 60 territories (i.e., 12 trials per treatment) in Mt. Austen, Guadalcanal Island (9°28.035'S, 159°58.452'E) from July 6 to July 12, 2007, and May 22 to May 26, 2008. For the white-capped *M. c. richardsii*, we tested 60 territories (i.e., 12 trials per treatment) on Tetepare Island (9°29.218'S, 159°59.230'E) from June 24 to June 30, 2007 and May 15 to May 20, 2008.

## Results

### MOLECULAR PHYLOGENY

A Bayesian phylogenetic analysis of the mitochondrial (*ND2* and *DII*) and nuclear intron (*TGFB2*) DNA sequence data produced a well-resolved phylogenetic hypothesis for the *M. melanopsis* superspecies complex (Fig. 2). ML analysis produced a tree with similar topology and relative support levels (see Supporting Fig. S2). The data support monophyly of a Solomon endemic clade embedded within the *M. melanopsis* superspecies complex. Among the Solomon endemics, chestnut-bellied *M. c. castaneiventris* individuals from Malaita form a basal clade sister to all other samples included in the analysis. Samples of Makira Island chestnut-bellied *M. c. megarhynchus*, and all-black *M. c. ugiensis* are interspersed such that neither taxon is monophyletic but together form a well-supported clade sister to a clade of the remaining Solomon endemics. Within this latter clade, the white-capped New Georgia endemics, *M. c. richardsii*, form a solidly monophyletic grouping within a large polytomy that includes nominate *M. c. castaneiventris* samples from three islands (Choiseul, Isabel, and Guadalcanal) and *M. c. erythrostickus* samples from Shortland Island (Bougainville). The *M. c. erythrostick-*

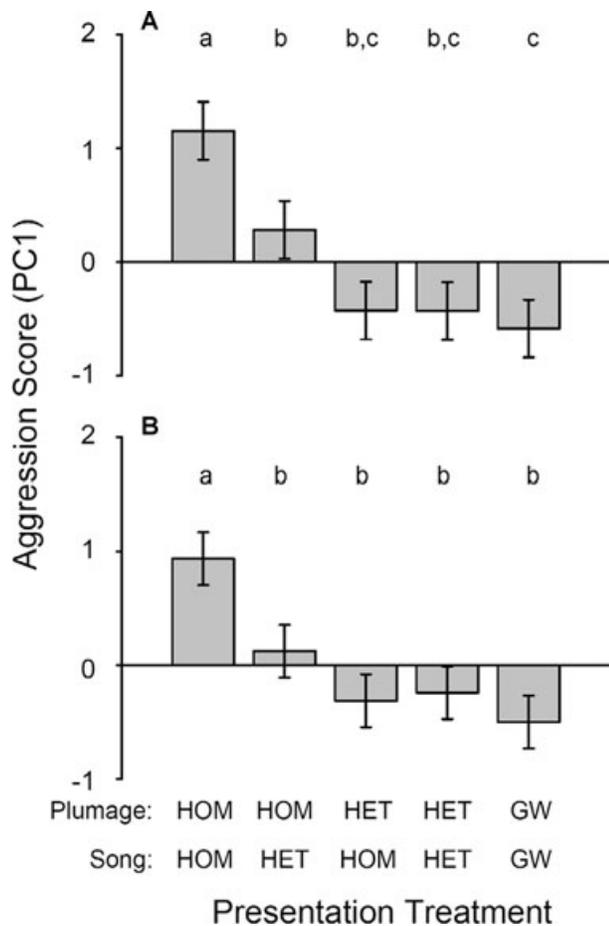
*tus* samples were intermixed with some Choiseul samples of *M. c. castaneiventris* in a shallow clade.

A LR test of rate homogeneity across the phylogeny indicated that the *ND2* data did not differ significantly from clock-like evolution ( $\chi^2 = 35.808$ ,  $df = 44$ , critical value = 60.481). Using the *ND2*-specific substitution rate of 0.02 to 0.022 substitutions per site per lineage million years (Arbogast et al. 2006), our phylogenetic analyses placed the most recent common ancestor of *M. castaneiventris* and *M. cinerascens* at just over half a million years (Myr) ago (range: 0.522–0.574 Myr ago; Fig. 2). The *M. castaneiventris* clade started diversifying within the Solomon Archipelago ca. 0.478 to 0.526 Myr ago, with the Malaita Island population of *M. c. castaneiventris* and the Makira Island clade (*M. c. megarhynchus* and *M. c. ugiensis*) diverging in rapid succession from the rest of the *M. castaneiventris* complex. The white-capped *M. c. richardsii* form diverged from its sister taxa more recently at ca. 0.298 to 0.328 Myr ago.

### EXPERIMENTAL TEST OF SPECIES RECOGNITION

PCA extracted four PC scores that explained 64.19% of variation in behavioral response to mounts and song playbacks (PC1 = 20.82%, PC2 = 19.46%, PC3 = 14.06%, PC4 = 9.85). Component loadings indicate the relationship between specific PC scores and the responses (Supporting Table S2): PC1 was positively associated with time spent on the mount's stick (0.88), number of attacks (0.79), and raspy calls within 2 m of the mount (0.79), and negatively associated with time spent in the canopy (−0.53); PC2 was positively associated with time spent in adjacent (< 2 m) vegetation, whistles within 2 m of the mount (0.75), and number of flights near the mount with no contact (0.74); PC3 was positively associated with whistles greater than 2 m from the mount (0.95) and time spent calling in the canopy (0.74); and PC4 was positively associated with chatter calls less than (0.55) and greater than (0.63) 2 m from the mount, and negatively associated with raspy calls greater than 2 m from the mount. PC1 is clearly associated with aggressive behavior (e.g., number of attacks) and negatively associated with behavior that indicated lack of general interest (e.g., time spent in the canopy), whereas PC2 is associated with general interest or inspection behavior (e.g., time spent in adjacent vegetation, number of flights by the mount). PC3 and PC4 indicate a more general response to the stimuli, with PC4 possibly indicating submissive or solicitation behavior. Because PC1 is the clearest index of aggressive response, we focus our analyses using PC1 as an index of recognition of sexual competitors.

We found that territory-owners ignored the golden whistler control, and responded most aggressively (i.e., highest PC1 scores) to the treatment of homotypic plumage and song, least aggressively to the heterotypic *M. castaneiventris* ssp. treatment, and intermediately to a mismatch between color and song type (Fig. 3). In the analysis that excluded the golden whistler trials,



**Figure 3.** Mean ( $\pm$ SE) aggression scores/response (PC1) of (A) chestnut-bellied *M. c. castaneiventris* ( $n=12$  trials per treatment) and (B) white-capped *M. c. richardsii* ( $n=12$  trials per treatment) territory-owners toward various combinations of plumage and song types (HOM=homotypic, HET=heterotypic *M. castaneiventris* ssp., GW=golden whistler/control). Positive scores indicate more intense aggressive response to the plumage and song types (conversely, negative scores mean lack of response). The SE shown is the least squares estimates of standard error.

the nested ANOVA revealed that plumage and song type influenced the intensity of aggressive response by territory-owners (Table 1). The ANOVA further indicated a significant interaction between plumage and song type but no significant taxon effect (Table 1). Time of day in which a trial was run did not affect PC1 ( $F_{1,78} = 2.50, P = 0.12$ ), and so time was not included in the final full ANOVA model. In the second analysis that included the golden whistler trials (Table 2), we similarly found a strong treatment (i.e., plumage and song type) effect. The linear contrast for the main effect of plumage and song type accounted for more than 80% of the variation explained by the main effect, and was highly significant (Table 2). Standard post hoc, pairwise comparisons show a similar pattern, with responses to homotypic plumage and song being different from the response to all other

**Table 2.** Mixed-model ANOVA of aggressive response (PC1) by territory-owners to taxidermic mount presentations and song playbacks, including the golden whistler (heterospecific control) trials. Probability values ( $P$ ) from parametric ANOVA and randomization tests (in parenthesis) are provided (model  $R^2$  for *M. c. richardsii* analysis = 0.304; *M. c. castaneiventris* = 0.374).

Subspecies/ Factor	df	Type III sum of squares	$F$	$P$
<i>M. c. richardsii</i>				
Treatment (plumage and song type)	4	15.55	6.02	<0.001 (<0.001)
Linear contrast	1	12.55	19.43	<0.001 (<0.001)
Residuals	55	35.53		
<i>M. c. castaneiventris</i>				
Treatment (plumage and song type)	4	25.45	8.24	<0.001 (<0.001)
Linear contrast	1	21.09	27.32	<0.001 (<0.001)
Residuals	55	42.46		

treatments. Note, that for both taxa, response to homotypic mount with homotypic song is significantly different from the response to homotypic mount with heterotypic song, suggesting that divergent song likewise influences the behavioral responses.

Of the 120 mount presentation and song playback trials we ran, 43 resulted in territory-owners approaching (<2 m) the mount. In all of these, at least two birds displayed aggression, with one individual taking the lead and approaching the mount, whereas a second bird typically calling from adjacent vegetation (or further away). In the dichromatic white-capped form, 14 of the 15 trials that resulted in an approach were initiated by an adult male, with the second bird, when visible, clearly a female (in one trial, a female approached and attacked before the adult male). In the monochromatic chestnut-bellied taxon, all of the 28 trials that resulted in territory-owners approaching the mount involved at least two birds. Unfortunately, identification of sex during the experiments was not feasible for this taxon, but, similar to the dichromatic taxon, one individual took the lead whereas a second bird called from adjacent vegetation (or further).

## Discussion

### EVOLUTIONARY HISTORY OF THE *MONARCHA CASTANEIVENTRIS* COMPLEX

The Solomon endemic *M. castaneiventris* complex forms a monophyletic clade, and evidence from this and earlier work suggest that signal divergence among island populations occurred recently and rapidly. Previous analyses of the entire radiation of Pacific monarchs indicate that the speciation events within the *M. melanopsis* superspecies complex occurred less than one million

years ago (Filardi and Moyle 2005; Filardi and Smith 2005). In support of this recent time frame, our molecular clock estimates suggest that the split between *M. castaneiventris* and its sister taxon, *M. cinerascens*, occurred about 500,000 years ago. Remarkably, the split between the white-capped, dichromatic *M. c. richardsii* and the nominate chestnut-bellied, monochromatic *M. c. castaneiventris* forms may have occurred as recently as 300,000 years ago. Further, the all-black *M. c. ugiensis* and Makira Island chestnut-bellied *M. c. megarhynchus* subspecies form a strong monophyletic clade but fail to form, respectively, monophyletic groups within their clade. A more detailed population genetics study using additional nuclear intron genes suggests that the retention of ancestral polymorphism caused by recent divergence and contemporary gene flow may explain the lack of reciprocal monophyly between these two subspecies (J. A. C. Uy, R. G. Moyle, C. E. Filardi, and Z. A. Cheviron, unpubl. ms.) Although a “universal” rate of molecular divergence in birds does not exist, the low level of divergence in *ND2* ( $<0.025$  *P*-distance within *M. castaneiventris*) is congruent with a recent burst of speciation (García-Moreno 2004; Filardi and Moyle 2005).

The phylogenetic structure of the *M. castaneiventris* complex, especially the paraphyly of the nominate form and the strong monophyly of *M. c. megarhynchus* and *M. c. ugiensis*, is best predicted by the geography of the Solomon Archipelago during the late Pleistocene. Because sea levels were approximately 200 m lower during the Pleistocene glacial maxima, many of the islands throughout the archipelago were likely to have been connected repeatedly by land bridges. This resulted in four major island groups: Makira Island, Malaita Island, the New Georgia group, and Greater Bukida, which was formed by the islands of Bougainville, Choiseul, Isabel, and Guadalcanal (Mayr and Diamond 2001). The phylogenetic pattern of the *M. castaneiventris* complex fits these major island groupings, with birds from Malaita, Makira, Greater Bukida, and the New Georgia group forming distinct clades (Fig. 2). The phylogeny thus suggests that geographic barriers, both historic and current, have a clear imprint on the phylogenetic structure of this group.

Because of their distinct plumage and sexual dichromatism, several authors consider the white-capped *M. c. richardsii* and Bougainville *M. c. erythrostickus* taxa as distinct allospecies (Mayr and Diamond 2001; Filardi and Smith 2005). Our phylogeny indicates that the white-capped taxon clearly forms a distinct monophyletic clade that diverged from its sister taxa about 300,000 years ago, providing support for its elevation to allospecies status. This makes sense because the New Georgia Islands have always been isolated from the rest of the Solomon Archipelago. In contrast, the Bougainville taxon *M. c. erythrostickus* fails to form a monophyletic clade and groups with *M. c. castaneiventris* samples from adjacent Choiseul Island. Choiseul and Bougainville are relatively close, and so there is potential for contemporary

gene flow between the two forms. Further, even in the absence of contemporary gene flow, Choiseul and Bougainville was connected during the last ice age, and so incomplete lineage sorting may account for the lack of reciprocal monophyly. Hence, phylogenetically, there is less support for considering this form as a distinct allospecies. Although there is evidence in support of elevating the white-capped form and, possibly, the Malaita Island *M. c. castaneiventris* form into allospecies level, we use Mayr's original taxonomic classification in this article (i.e., subspecies of a variable complex) for simplicity.

With the exception of the Makira Island populations, most of the *M. castaneiventris* forms sing a simple whistle note that varies in note duration (Fig. 1). In contrast, plumage color is far more variable and is the most distinct between sister taxa. For instance, between the white-capped and nominate chestnut-bellied sister taxa pair, plumage variation is qualitative (i.e., presence vs. absence of white cap, and mono- vs. dichromatism), whereas variation in songs is more limited (e.g., only in length of whistle note; see Supporting Fig. S1), suggesting that plumage color is evolving more rapidly than song structure between taxa. The more rapid divergence of color over song structure suggests that plumage differences may be more important in homotypic recognition and premating isolation between sister taxa.

## MULTIMODAL SIGNAL DIVERGENCE AND SPECIATION

With our phylogenetic analyses indicating rapid divergence of plumage color and song among island endemics, we then tested for the roles of divergent multimodal signals in species recognition with the expectation that the more variable plumage color should be more important than song structure. Our behavioral experiments indicate that male and female territory-owners ignored the heterospecific golden whistler control and responded more strongly to homotypic than to heterotypic plumage and song types, indicating that individual birds use divergent plumage and song in conspecific/homotypic recognition (e.g., Baker and Baker 1990). Note, that the use of the heterospecific control attempts to distinguish between an aggressive response to an ecological and sexual competitor. The lack of response to the golden whistler stimuli suggests that the aggressive response to the homotypic stimuli is likely a response to a sexual rather than an ecological competitor. Therefore, our results are consistent with the hypothesis that divergent plumage color and song structure are used in the recognition of sexual competitors.

Our analyses, however, also reveal that divergent plumage color is more important in homotypic recognition than divergent song (Table 1), which is consistent with a parallel study testing the Makira Island chestnut-bellied *M. c. megarhynchus* and Santa Ana Island all-black *M. c. ugiensis* forms (J. A. C. Uy, R. G. Moyle, C. E. Filardi, and Z. A. Cheviron,

unpubl. ms.) Other studies show that the level of homotypic/species recognition is influenced by the extent of differences in signals between taxa (e.g., Nelson 1989; Coulridge and Alexander 2002; but see Bernal et al. 2007). Signals that are more variable between taxa should be more useful in recognition, and our results indicating a stronger plumage than a song effect are consistent with this. However, divergent song also plays a role, as our post-hoc comparisons and linear contrast show a significant reduction in response to homotypic plumage when accompanied by heterotypic songs (Fig. 3, Table 2).

Assuming that traits used in species recognition are also used in mate choice, as has been confirmed in several studies (e.g., Baker and Baker 1990; Baker 1991; Patten et al. 2004), our experiments suggest that changes in multimodal signals contribute to pre-mating reproductive isolation. This is consistent with several broad-scale comparative studies (e.g., Barraclough et al. 1995; Cardoso and Mota 2008; Seddon et al. 2008; but see Owens et al. 1999; Phillimore et al. 2006), and comparisons of closely related taxa (e.g., Seehausen and van Alphen 1998; Gray and Cade 2000; Uy and Borgia 2000; Boughman 2001; Irwin et al. 2001; Seddon and Tobias 2007; Filardi and Smith 2008) that provide support for an important role of mating signal divergence in species formation. Most studies that link signal divergence and speciation between closely related taxa, however, test only a single signal or group of signals of the same sensory modality (but see Baker and Baker 1990; Patten et al. 2004; Mullen et al. 2007). Our work highlights the importance of exploring the evolutionary consequences of divergent multimodal signals, as many taxa differ in multimodal display traits (Rowe 1999; Candolin 2003) and, by not explicitly testing for the effects of divergent multimodal signals, the use of divergent signals in conspecific recognition may remain undetected or inferred to be weaker (Baker and Baker 1990). For instance, our work indicates that the treatment with homotypic plumage and song elicited a stronger response than the treatments with a mismatch in plumage and song types. These results are consistent with two other avian studies in which the combination of homotypic song and plumage elicited a stronger response from females than a mismatch in song and plumage, indicating that both signals are important in conspecific recognition and mate choice (Baker and Baker 1990; Patten et al. 2004).

The advantages of using multimodal over unimodal signals may be driven by the signaling environment and/or receiver psychology (Rowe 1999). With respect to the physical environment, each signal modality has unique advantages and disadvantages because of the unique properties of signals and how they interact with the physical environment. For instance, acoustic signals can be transmitted long distances and can propagate around obstructions, and so acoustic signals are ideal for long-range communication. In contrast, visual signals are limited by body size and can be obstructed easily, and so visual signals may be more ideal

for short-range communication (see Bradbury and Vehrencamp 1998). The use of multimodal signals in social interactions may therefore facilitate conspecific recognition by allowing receivers to assess the signaler in a hierarchical process at various distances (e.g., Mullen et al. 2007). If, for instance, both acoustic and visual signals are used, then receivers can first assess the signaler from a distance then continue the assessment at a closer range after initial decisions have been made (e.g., McDonald 1989; Gibson 1996). In the white-capped *M. c. richardsii* form, Filardi and Smith (2008) found that territorial males preferentially attacked adult male mounts and courted female mounts. Similarly, in our study, plumage played a more critical role than song type in eliciting aggression in both taxa (see also J. A. C. Uy, R. G. Moyle, C. E. Filardi, and Z. A. Cheviron, unpubl. ms.) Plumage color therefore clearly plays a critical role in close-range recognition of sexes and conspecifics. Whistles, on the other hand, may influence the initial response, functioning as long-range advertisement signals. In a playback experiment, a territory-owner's latency to respond to a stimulus can be used as an assay of long-distance cueing. That is, if resident birds respond quicker (e.g., vocal response) to a homotypic than a heterotypic song type, then this can be interpreted as birds using divergent songs in long-distance communication. Unfortunately, in our experiments, we found territories by listening for calling birds; hence, territory-owners were typically calling at the start of the experiment and so the latency of response to our test stimuli could not be measured accurately. Additional experiments are needed to assess how each signal type is used in conspecific recognition; however, our results indicating reduced response to a mismatch in plumage and song suggest that both signal types are important in the recognition of sexual competitors.

In addition to facilitating conspecific assessment, the use of multimodal signals may also ensure efficient communication in a variable habitat (Candolin 2003). For instance, visual conditions in streams and lakes may change with rainfall or pollution (e.g., increased turbidity), making visual signals less effective (Seehausen et al. 1997; Engström-Öst and Candolin 2007). The use of an alternative signal of a different sensory modality, like olfactory signals (Rafferty and Boughman 2006), may allow individuals to continue communicating effectively when conditions are no longer favorable for one signal type. With respect to signal divergence and speciation, if indeed multimodal signals can ensure the effectiveness of signals in a broader range of conditions then perhaps multimodal signal divergence can more rapidly promote speciation and/or maintain species boundaries.

Finally, once received, multimodal signals may be more effective than unimodal signals in eliciting a response from the receiver. This is because the use of multiple components may result in reducing the reaction time of receivers by lowering the required threshold in which detection occurs (reviewed by Rowe

1999). This is consistent with our results indicating a stronger response to homotypic mounts and songs than a mismatch of the two. Further, multimodal signals may enhance associative learning (Rowe 1999). For instance, in learning to navigate through a maze, rats *Rattus norvegicus* who were trained with both visual and acoustic cues learned quicker than those trained only with visual cues (Eninger 1952). In birds in which individuals likely learn or imprint on preferences for conspecifics through association (reviewed by Price 2007), imprinting on multimodal signals may enhance homotypic recognition and assortative pairing, which, in turn, may accelerate that evolution of premating reproductive isolation. Future work should test if rates of speciation among clades vary depending on the number of signals and, more importantly, signal types diverging.

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## Supporting Information

The following supporting information is available for this article:

**Figure S1.** Quantitative comparison of whistle note parameters for the chestnut-bellied *Monarcha castaneiventris* and white-capped *M. c. richardsii* color forms.

**Figure S2.** Phylogeny of the *Monarcha melanopsis* superspecies complex inferred from Maximum Likelihood (ML) analysis.

**Table S1.** Taxon sampling for the island groups used in our study. Asterisks indicate taxa included in Filardi and Smith (2005).

**Table S2.** Component factor loadings (correlation matrix) for the four PC scores and 12 behavioral categories.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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SUPPLEMENTARY MATERIALS

Supplementary Table S1: Taxon sampling for the island groups used in our study. Asterisks indicate taxa included in Filardi and Smith (2005).

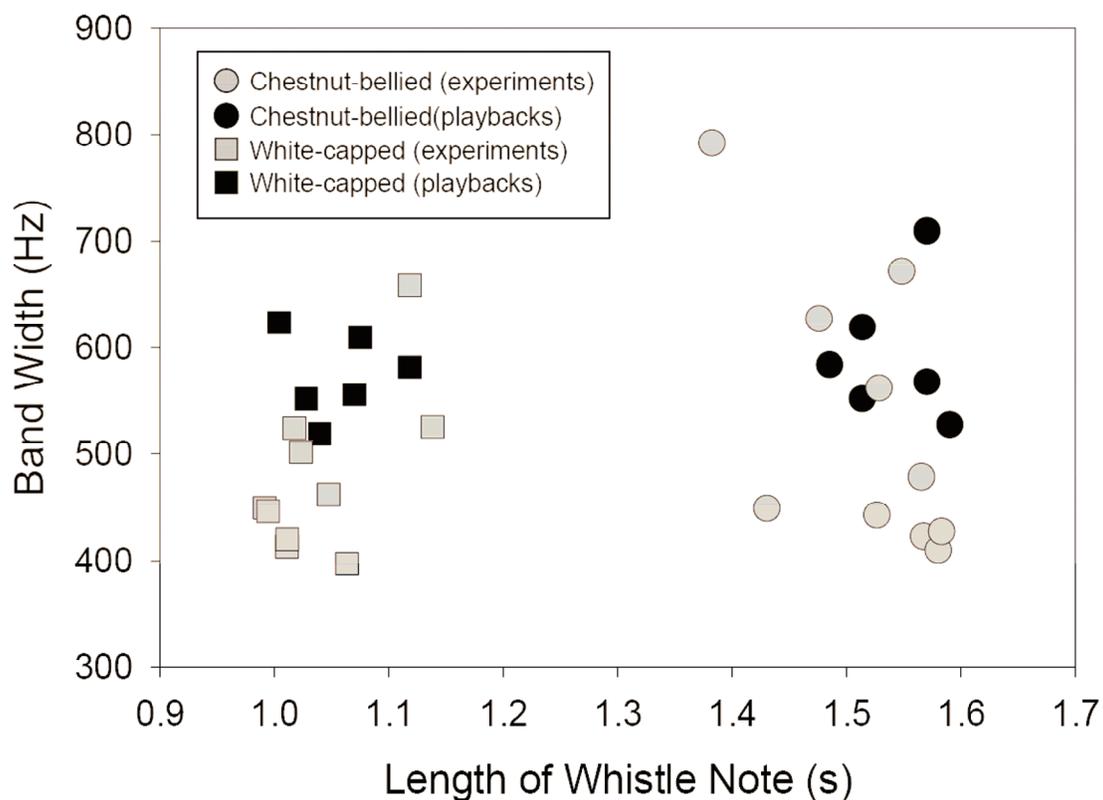
<b>Species</b>	<b>Locality</b>	<b>n</b>	<b>Specimen vouchers<sup>a</sup></b>
<i>M. c. castaneiventris</i> *	Choiseul Island	5	UWBM 63080, UWBM 63168, UWBM 63180, UWBM 63186, UWBM 63187
<i>M. c. castaneiventris</i> *	Isabel Island	4	UWBM 58768, UWBM 60221, UWBM60290, UWBM 60307
<i>M. c. castaneiventris</i> *	Guadalcanal Island	5	UWBM 60262, UWBM 60270, UWBM60345, UWBM 60353, UWBM 60363
<i>M. c. castaneiventris</i> *	Malaita Island	3	UWBM 66030, UWBM 66032, UWBM66092
<i>M. c. megarrhynchus</i>	Makira Island	3	unvouchered blood samples <sup>b</sup>
<i>M. c. ugiensis</i>	Ugi Island	1	unvouchered blood samples <sup>b</sup>
<i>M. c. ugiensis</i>	Santa Ana Island	5	unvouchered blood samples <sup>b</sup>
<i>M. c. erythrostickus</i>	Shortland Island	2	unvouchered blood samples <sup>b</sup>
<i>M. c. richardsii</i> *	New Georgia Group	10	UWBM 63020, UWBM 66003, UWBM66019, UWBM 66021, UWBM 66081, UWBM 68079, UWBM 68080, UWBM69789, and unvouchered blood samples <sup>b</sup>
<i>M. frater</i> *	New Guinea	1	VM (E034)
<i>M. melanopsis</i> *	Australia	3	UWBM 62890, ROM(JB954), ROM(AJB5629)
<i>M. cinerascens</i> *	SI islets	3	UWBM 60242, UWBM 60337, UWBM 67947
<i>Chytorhynchus hamlini</i> *	Rennell Island	1	UWBM 69806

<sup>a</sup>ROM, the Royal Ontario Museum, UWBM, University of Washington Burke Museum; VM, Museum of Victoria (Australia).

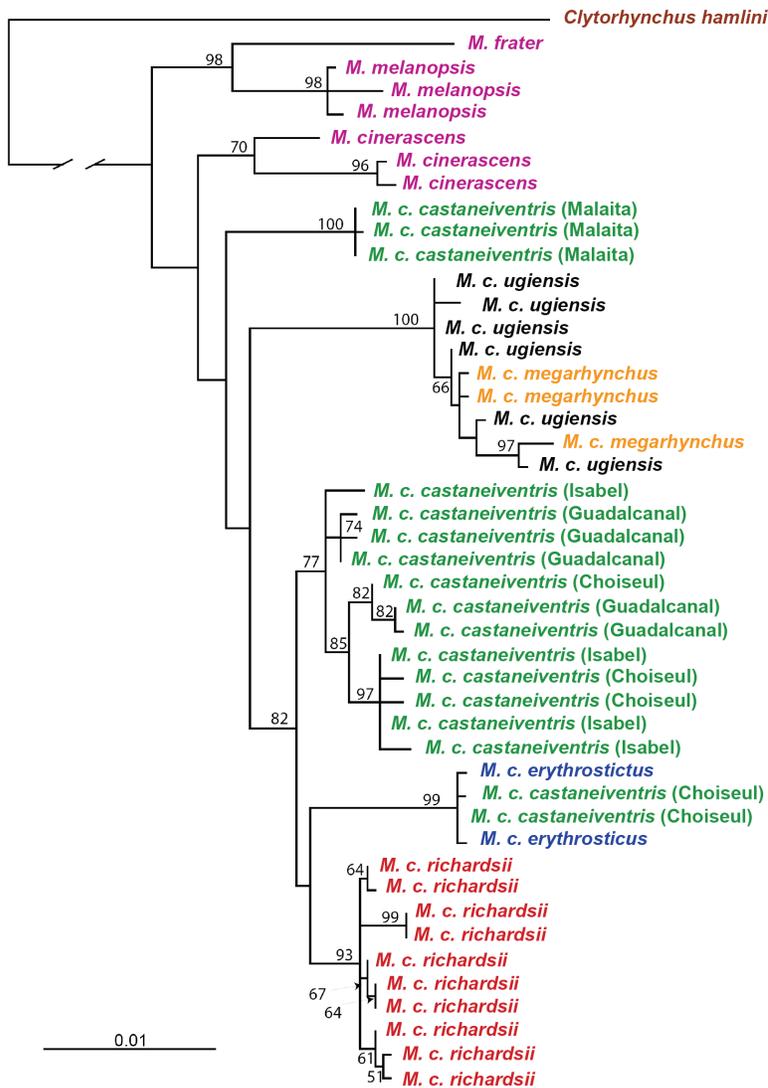
<sup>b</sup>These individuals were part of a banding study and thus not sacrificed for voucher specimens. Similar species do not occur on these islands and so identification was straightforward.

Supplementary Table S2: Component factor loadings (correlation matrix) for the four PC scores and 12 behavioral categories.

<b>Behavioral Response</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>
Number of attacks	0.787	0.104	-0.129	0.206
Time spent in canopy	-0.533	-0.634	-0.079	0.045
Time spent emitting raspy calls < 2m from mount	0.785	0.036	-0.163	-0.055
Time spent emitting chatter calls < 2m from mount	0.226	0.263	-0.200	0.546
Time spent emitting whistles < 2m from mount	0.174	0.753	-0.057	-0.165
Time spent emitting calls in canopy	-0.221	-0.218	0.735	-0.046
Time spent emitting raspy calls > 2m from mount	-0.127	0.000	-0.300	-0.609
Time spent on mount's perch	0.881	0.207	-0.136	0.006
Time spent on adjacent vegetation	0.128	0.804	-0.187	0.145
Time spent emitting chatter calls > 2m from mount	-0.129	-0.075	-0.080	0.634
Time spent emitting whistles > 2m from mount	-0.143	-0.030	0.947	0.028
Number of flights by mount, with no contact	-0.033	0.736	-0.085	0.111



Supplementary Figure S1: Comparison of whistle note parameters for the chestnut-bellied *M. c. castaneiventris* (circles) and white-capped *M. c. richardsii* (squares) color forms. For this comparison, we use data from the 12 recordings used for the playback experiments (black squares and circles) and 20 recordings from territory-owner responses during our experiments (gray squares and circles). Each data point represents the average measure of five to ten whistle notes from a unique territory/recording. Because recordings of responses during our experiments were poorer in quality (e.g., no external microphone), we could only reliably estimate and compare the following characteristics: note length (s), band width (Hz), minimum frequency (Hz), maximum frequency (Hz), and the frequency at which the maximum power occurs. These parameters were measured using Raven 1.2. Only note length (Mean  $\pm$  S.E.: chestnut-bellied,  $1.526 \pm 0.015$  s; white-capped,  $1.047 \pm 0.012$  s) is different significantly between taxa (one-way ANOVA,  $F_{1,30} = 654.90$ ,  $p \ll 0.001$ ). Note that the recordings used for the playback experiments fall well within the variation for each taxon, indicating that the recordings effectively represent the song structure of each taxon.



Supplementary Figure S2: Phylogeny of the *Monarcha melanopsis* superspecies complex inferred from Maximum Likelihood (ML) analysis. Divergent taxa are color-coded as in Fig. 2: nominate chestnut-bellied form (*M. c. castaneiventris*) in green, Makira Island chestnut-bellied form (*M. c. megarhynchus*) in grey, all-black form (*M. c. ugiensis*) in black, white-capped form (*M. c. richardsii*) in red, and Bougainville form (*M. c. erythrostickus*) in blue. The three species that are not endemic to the Solomon Islands (*M. cinerascens*, *M. frater*, *M. melanopsis*) but are part of the superspecies complex are in purple font. Based on a more general *Monarcha* phylogeny that involved 14 species distributed throughout Australasia and the South Pacific (Filardi and Smith 2005), we used the Rennell Shrikebill *Clytorhynchus hamlini* (brown font) as the outgroup. Nodal support based on ML bootstrap values are provided above each branch. For our analyses, we used the TrN+I+G model of nucleotide substitution.