

CAN SONG DISCRIMINATE BETWEEN MACGILLIVRAY'S AND MOURNING WARBLERS IN A NARROW HYBRID ZONE?

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Abstract. Areas where divergent taxa come into secondary contact are important in identifying the reproductive barriers that may have evolved between them. Here we investigate whether song variation plays a role in maintaining a recently described hybrid zone between MacGillivray's (*Oporornis tolmiei*) and Mourning Warblers (*O. philadelphia*) in northeastern British Columbia, Canada. We analyzed song recordings and genetic data from across the ranges of both species as well as where they occur in sympatry. We predicted that if song differences are an important pre-mating reproductive barrier between these taxa, song and genotype in the hybrid zone should covary. Using a discriminant function analysis, we show that the two species' songs are differentiated in allopatry but that songs converge in sympatry, with a weak and nonsignificant association between songs and diagnostic mitochondrial and nuclear genetic markers in the hybrid zone. These results suggest that song divergence is unlikely to be an important reproductive barrier between these taxa and that learning of the other species' song may be promoting continued hybridization. This result contrasts with studies of other species pairs that maintain song differences in sympatry and come into contact in this same geographic region.

Key words: hybrid zone, hybridization, *Oporornis philadelphia*, *Oporornis tolmiei*, signal convergence, song, speciation.

¿Puede el Canto Discriminar entre *Oporornis tolmiei* y *O. philadelphia* en una Estrecha Zona de Hibridación?

Resumen. Las áreas donde taxones divergentes entran en contacto secundario son importantes para identificar barreras reproductivas que pueden haber evolucionado entre ellos. Aquí, investigamos si la variación en el canto juega un papel en el mantenimiento de una zona de hibridación recientemente descrita entre *Oporornis tolmiei* y *O. philadelphia* en el noreste de la Columbia Británica, Canadá. Analizamos grabaciones de canto y datos genéticos de todo el rango de distribución de ambas especies, así como de las áreas en que se encuentran en simpatria. Predijimos que si las diferencias entre los cantos son una barrera reproductiva importante entre estos dos taxones, el canto y los genotipos en la zona de hibridación deberían covariar. Utilizando un análisis de función discriminante, mostramos que los cantos de las dos especies son diferentes en alopatría, pero que éstos convergen en simpatria, con una asociación débil y no significativa entre los cantos y los marcadores genéticos mitocondriales y nucleares en la zona de hibridación. Estos resultados sugieren que es poco probable que la divergencia del canto represente una barrera reproductiva importante entre estos taxones y que el aprendizaje del canto de la otra especie puede ser lo que promueve la continua hibridación en esa zona. Este resultado contrasta con los de estudios de otros pares de especies que mantienen diferencias en sus cantos en simpatria y que entran en contacto en esta misma región geográfica.

INTRODUCTION

A central question in the study of speciation is whether reproductive barriers between divergent taxa are caused by (1) divergence in traits associated with mate choice (i.e., pre-mating isolation) or (2) reduced fitness of hybrid offspring (i.e., post-mating isolation; Coyne and Orr 2004, Price 2008). Hybrid zones between closely related species present a unique opportunity for study of partial reproductive barriers because they contain novel genetic and phenotypic combinations that can be assayed in a similar geographic environment (Barton 1983, Rieseberg and Buerkle

2002, Dalziel et al. 2009). Especially in birds, the role of song as a reproductive barrier has been informed by such studies of secondary contact, hybridization, and range overlap (Robbins et al. 1986, Sætre et al. 1997, Irwin 2000, de Kort et al. 2002, Secondi et al. 2003, Haavie et al. 2004, Gee 2005, den Hartog et al. 2007, Toews and Irwin 2008).

Song is one of the principal avian mating signals, used primarily by males to advertise themselves as potential mates to conspecific females (Catchpole and Slater 1995, Price 2008). Through this mate-attraction function, song is an important means of generating and maintaining reproductive

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isolation between species (Catchpole and Slater 1995, Irwin 2000). Most species of songbirds have both learned and genetic components to their vocalizations, which, along with the numerous ecological and social pressures on song composition and structure, have the potential to produce complex dynamics upon secondary contact (Slabbekoorn and Smith 2002, Forstmeier 2009, Tobias and Seddon 2009). For instance, song convergence might be expected if signals are constrained by the acoustic environment in sympatry (Secondi et al. 2003) or if there is indiscriminate learning of heterospecific song (Robbins et al. 1986). Alternatively, if selection against hybrids is strong, divergence of signals in sympatry might be expected from character displacement or reinforcement (Sætre et al. 1997, Haavie et al. 2004). Finally, unique combinations of the parental species' vocal characteristics in hybrids could generate novel songs that have varying effectiveness within and outside of the contact zone (den Hartog et al. 2007). Given these diverse outcomes, there is debate as to whether the characteristics of song evolution are more likely to cause song to contribute to or detract from the accumulation of reproductive isolation between diverging populations (Slabbekoorn and Smith 2002, Lachlan and Servedio 2004, Price 2008). Distinguishing between these alternatives within avian hybrid zones can be used to understand the evolutionary history of certain groups and, more generally, help resolve the relationship between bird song and speciation.

Irwin et al. (2009) discovered a zone of hybridization of the MacGillivray's (*Oporornis tolmiei*) and Mourning (*O. philadelphia*) Warblers in northeastern British Columbia, Canada. This hybrid zone occurs in an area where a number of other west-east species pairs also come into contact, including the Pacific Wren (*Troglodytes pacificus*) and Winter Wren (*T. troglodytes*; Toews and Irwin 2008), the Audubon's Warbler (*Dendroica [coronata] auduboni*) and Myrtle Warbler (*D. [c.] coronata*; Brelsford and Irwin 2009), and the Townsend's Warbler (*Dendroica townsendi*) and Black-throated Green Warbler (*D. virens*; Toews et al., in press). The MacGillivray's and Mourning Warbler hybrid zone is centered 80 km east of the crest of the Rocky Mountains and is ~130 km wide (Irwin et al. 2009). The narrowness of this hybrid zone relative to the presumed dispersal of these warblers (approximately 30 km per generation, as estimated in closely related species; Rohwer and Wood 1998) suggests that some form of selection maintains the zone.

Previous studies of mitochondrial DNA suggest that MacGillivray's and Mourning Warblers diverged in allopatry approximately 1.1 million years ago, when they were confined to western and eastern glacial refugia (Mengel 1964, Weir and Schluter 2004). Most notable among their distinguishing characteristics are the white eye arcs of the adult MacGillivray's, rarely found in the Mourning, and the darker breast patch of the Mourning Warbler (Pitocchelli 1990, 1992, 1993, 1995). These taxa are similar in their affinity for mixed deciduous habitat with dense undergrowth and moderate cover, although their breeding ranges are distinct: MacGillivray's Warbler typically breeds in and to the west of the Rocky Mountains,

the Mourning to the east of the Rockies throughout the boreal forest (Fig. 1; Pitocchelli 1990, 1993, 1995, Irwin et al. 2009).

Pitocchelli (1990) found that a multivariate analysis could be used to discriminate between the songs of these taxa, at least where they are allopatric. He speculated that the species may mate assortatively on the basis of song, although he recognized that testing this idea would require finding regions where both species occur in sympatry. Here, we seek to understand whether song is an important reproductive barrier between MacGillivray's and Mourning Warblers by studying song variation across the recently described area of overlap. To do this we first used a combination of song characteristics to distinguish between songs of birds in allopatry. We then asked how these song characteristics varied across the hybrid zone. We were especially interested in whether there are intermediate or novel song types in the zone of sympatry lacking in allopatry. Finally we tested whether songs in the hybrid zone are correlated with genetic markers and phenotypic traits that are diagnostic for the taxa. We analyzed song recordings from across the two species' breeding ranges, including those of

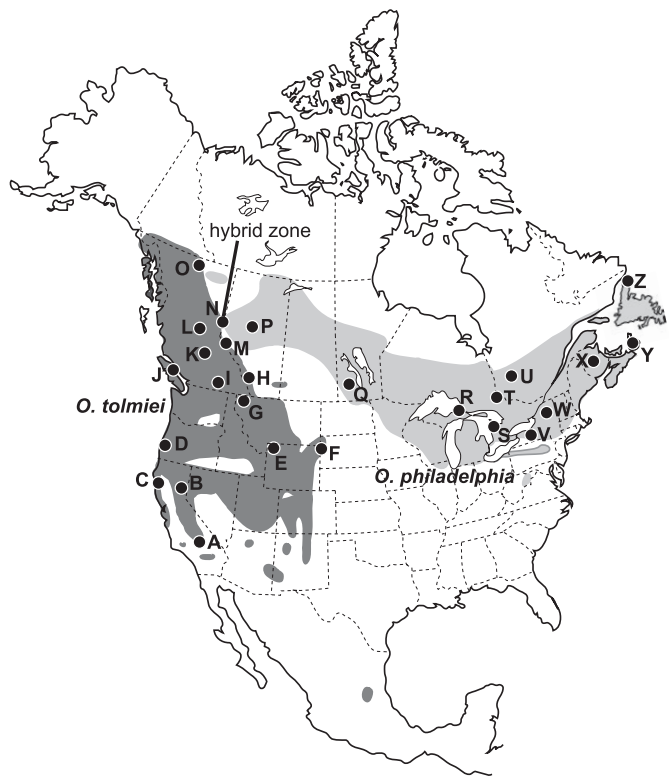


FIGURE 1. Breeding distributions of MacGillivray's (*Oporornis tolmiei*) and Mourning (*O. philadelphia*) Warblers in North America, along with the locations of sites of recordings used in this study (adapted from Pitocchelli 1993, 1995). Recordings from locations A–G, J, K, and Q–Z were obtained from the Macaulay Library, those from locations H, M, and O were made by J. Pitocchelli, and those from locations I, L, and N were made by DEI. One recording from location P was obtained from the Macaulay Library, while the rest were made by DEI.

individuals from within the contact zone, and predicted that if assortative mating by song is an important pre-mating reproductive barrier, then it should be a good predictor of an individual's genotype in the hybrid zone. This prediction results from the fact that there must be an association between a trait and genetic background for that trait to cause reproductive isolation between two genetically divergent forms, even for a trait such as song with both learned and genetic components.

MATERIALS AND METHODS

RECORDING ACQUISITION

We obtained recordings of songs of 79 birds from several sources. The majority of birds, including 30 from within the contact zone and 15 from areas of allopatry, were recorded by DEI in 2006, 2007, and 2008 (Fig. 1). Here we define "allopatric" populations as those occurring at least 200 km from the center of contact zone where Irwin et al. (2009) reported little genetic introgression. We obtained six recordings of allopatric populations from J. Pitocchelli, the remaining 28 from the Cornell Lab of Ornithology's Macaulay Library of Natural Sounds (Fig. 1).

GENETIC AND MORPHOLOGICAL DATA

To obtain measurements and blood samples for genetic analysis, we captured birds recorded by DEI in mist nets (see Irwin et al. 2009 for detailed methods). Each bird was genotyped for a Z-linked nuclear single-nucleotide polymorphism (in the CHD1Z gene) and a mitochondrial single-nucleotide polymorphism (in the COI gene), in both of which allopatric populations of the two taxa show nearly fixed differences (Irwin et al. 2009). We assumed these markers to be good indicators of an individual's overall genetic background; this assumption is supported by the fact that the transition zones of these markers line up closely with each other and with morphological traits (Irwin et al. 2009). As we captured only males, there were three possible combinations of alleles (two nuclear CHD1Z alleles and one mitochondrial COI allele) that could be identified as typical of the Mourning or MacGillivray's. We used these genotypes, presented by Irwin et al. (2009), to test the association between genotype and song. We scored eye arcs as score of zero for strong and one for none (Irwin et al. 2009). We also calculated the difference between the measured wing length and tail length (wing – tail), a trait distinguishing the two species (Irwin et al. 2009).

Genetic information was not available for the birds whose recordings we obtained from J. Pitocchelli or the Cornell Lab of Ornithology Macaulay Library. These birds, however, were all recorded well outside of the contact zone, so we assumed them to be either pure MacGillivray's Warblers or pure Mourning Warblers from their location.

SONG ANALYSIS

HLK created spectrograms of all recordings with RAVEN Interactive Song Analysis Software (Cornell Laboratory of

Ornithology, version 1.3). Each song recorded from an individual bird was numbered sequentially according to its place in the recordings. Songs with loud noise or other songs in the background were not included. From each bird recorded we selected three songs at random. For the four birds from which only two songs had been recorded, we used both songs. We chose a sample size of three because the songs of one individual typically vary little (Pitocchelli 1990). We followed Pitocchelli's (1990) definitions of a *note* as a single stroke on the spectrogram, representing a continuous sound, a *syllable* as a combination of notes repeated as a unit, and a *song* as a repeated group of syllables.

Using RAVEN, we measured 16 variables from each selected song (Fig. 2). For each bird, we calculated means from measurements of the three songs. To ensure that all variables would have equal weighting in a multivariate analysis we corrected for differences in units across variables by transforming each of the 16 variables so that each had a mean of zero and a standard deviation of one (across all birds in the study).

STATISTICAL ANALYSES

For each of the 16 song variables, we compared allopatric MacGillivray's and Mourning Warblers with a Mann–Whitney *U*-test in JMP 8 (SAS 8.0.2.2). We used this nonparametric test because some of the variables were not normally distributed. To find the axis of variation that best distinguishes the species in allopatry we used R for a linear discriminant function analysis (DFA) using individual means of the 16 song variables. We used three methods to determine how well our data from areas of allopatry classified an individual as either a MacGillivray's or Mourning singer. First, we applied the classification function from this DFA to each of the allopatric birds to determine the rate of misclassification. As this validation method is overly optimistic, we also performed the DFA on half of the allopatric individuals chosen at random and applied the resulting classification function to the remaining individuals to determine how well the function identified individuals in a separate sample of birds already identified genetically as pure MacGillivray's or Mourning. Finally, we used the jackknife method in R to see how the analysis performed with the sequential deletion of individual data points. Using the discriminant function based on all allopatric individuals, we then determined how well each of the individuals in the hybrid zone could be classified as either MacGillivray's or Mourning on the basis of its song characteristics.

A complicating factor in this analysis is that some individuals ($n = 14$) sang songs that had only a single syllable type, making it impossible to measure the two variables that refer to the frequency of part II of the song (variables 15 and 16). As a result we conducted two DFAs, one that included all individuals but excluded these two variables (we present this analysis here), another that included all variables but excluded individuals whose song lacked part II (presented in Appendix 1). The two approaches produced results that were generally consistent.

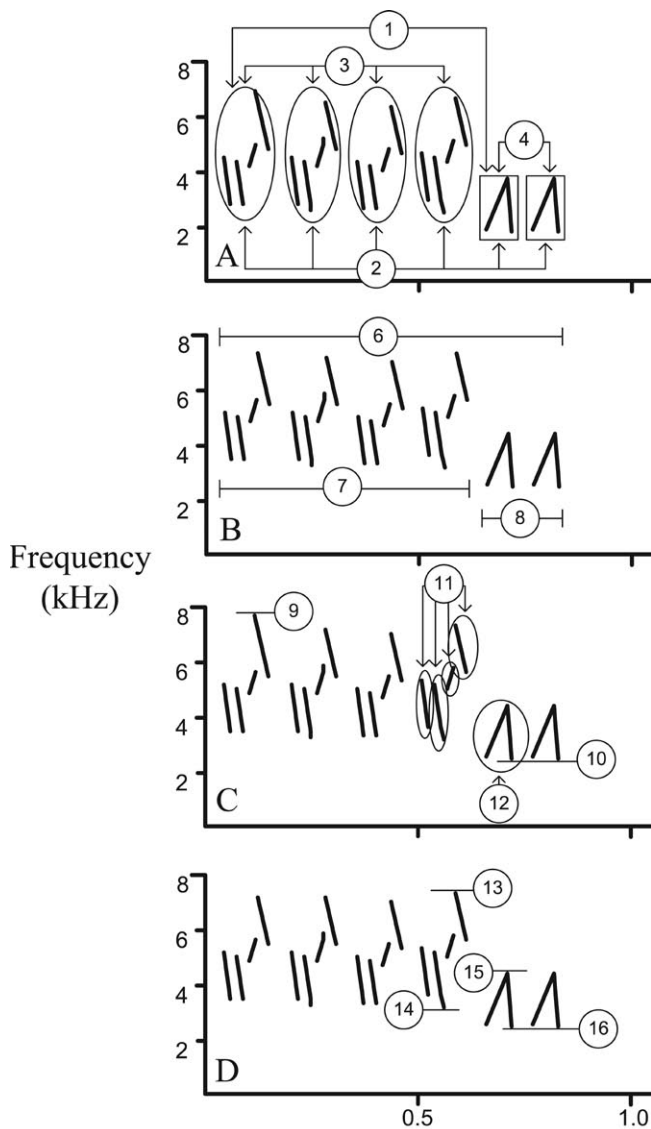


FIGURE 2. Sixteen of the variables measured marked on a simplified illustration of a song spectrogram: (A) 1, number of unique syllable types within the song; 2, total number of syllables per song; 3, number of repetitions of the first syllable type; 4, number of repetitions of the second syllable type; (B) 6, song duration (sec); 7, duration of part I, the length of time (sec) in the song during which the first syllable type is repeated; 8, duration of part II, the length of time (sec) in the song during which the second syllable type is repeated; (C) 9, the maximum frequency (Hz) over the entire song; 10, the minimum frequency (Hz) over the entire song; 11, number of notes in the last syllable of part I; 12, number of notes in the first syllable of part II; (D) 13, the maximum frequency of the last syllable of part I; 14, the minimum frequency of the last syllable of part I; 15, the maximum frequency (Hz) of the first syllable of part II; 16, the minimum frequency (Hz) of the first syllable of part II. A sixteenth variable, variable 5, number of repetitions of the third syllable type, is not shown here, as there is no third syllable in this example.

To compare how the DFA describes each of the three groups (MacGillivray's, Mourning, and hybrid zone), we performed a Kruskal–Wallis test analysis of variance by ranks in

R. We did this because the output of the DFA met the assumptions of equal variance but not normality (see below). For post hoc comparisons we used Bonferroni-corrected ($\alpha = 0.05/3$) Mann–Whitney U tests.

We used two methods to test for a correlation between a bird's song type and genotype in the hybrid zone. First, we regressed the discriminant classification score of birds in the hybrid zone against genotype (expressed as the number of eastern alleles, from 0 to 3) and calculated a Spearman correlation coefficient. Second, we used the binomial classifications from the DFA, which assigned individuals to MacGillivray's or Mourning Warbler song categories, in a generalized linear model. We performed a logistic regression using the "logit" link function in R to test whether the posterior assignment output (response variable) of the DFA was associated with an individual's genotype (explanatory variable). We also regressed the discriminant classification score of individuals' songs in the hybrid zone against morphological characteristics, namely, eye-arc score and wing–tail, and calculated Spearman correlation coefficients.

RESULTS

Although the songs of individual birds differed noticeably, each individual sang only one distinct song type (composed of the same syllable types) across all recordings. Some individuals varied in the number of repetitions of a syllable type from song to song and occasionally omitted the final syllable type from some songs. MacGillivray's Warblers typically sing songs that are more complex (more syllable types, more notes per syllable, and longer duration) and higher in frequency than those of Mourning Warblers (Table 1, Fig. 3). The DFA differentiated the allopatric MacGillivray's and Mourning songs, although there is some overlap between the groups (Fig. 4). The classification function derived from the DFA based on all of the allopatric individuals yielded a misclassification rate of 10% (Fig. 5). When we used half of the allopatric individuals as a training dataset the rate of misclassification of known singers was 12%. Finally, when we used the jackknife method of sequential removal of individual data points the misclassification rate rose to 27%.

There was a significant difference in the discriminant scores of the groups (Mourning, MacGillivray's, and hybrid zone; $\chi^2 = 32.2$, $df = 2$, $P < 0.0001$, Kruskal–Wallis nonparametric test). In a post hoc analysis, we found each of the groups to be significantly different from the others: MacGillivray's vs. Mourning ($U = 27.76$, $df = 1$, $P < 0.0001$), MacGillivray's vs. hybrid zone ($U = 14.07$, $df = 1$, $P = 0.0002$), and Mourning vs. hybrid zone ($U = 6.32$, $df = 1$, $P = 0.01$). The songs of birds in the hybrid zone fall within the natural variation observed in the allopatric populations, providing no evidence of the existence of a novel song type (Fig. 4). The posterior probability of assignment of hybrids' songs (Fig. 5) as either a MacGillivray's or Mourning Warbler was intermediate (average probability of assignment as MacGillivray's song = 0.44) between that of allopatric MacGillivray's (average probability of assignment

TABLE 1. Means, standard deviations, Mann–Whitney *U*-tests, and factor loadings from discriminate function analysis for 14 variables of allopatric MacGillivray's ($n = 23$) and Mourning Warbler song ($n = 26$).

| Variable | Allopatric MacGillivray's Warblers | | Allopatric Mourning Warblers | | Mann–Whitney <i>U</i> -test (df = 1) | | DFA factor loadings |
|---|------------------------------------|--------|------------------------------|--------|--------------------------------------|----------|---------------------|
| | Mean | SD | Mean | SD | <i>U</i> | <i>P</i> | |
| 1. Number of syllable types | 2.1 | 0.3 | 1.8 | 0.6 | 4.3 | 0.04 | 0.338 |
| 2. Total number of syllables per song | 7.3 | 1.0 | 6.4 | 1.5 | 5.0 | 0.03 | −0.875 |
| 3. Number of repetitions of first syllable | 4.6 | 0.7 | 4.8 | 1.1 | 0.4 | 0.50 | 0.290 |
| 4. Number of repetitions of second syllable | 2.7 | 0.9 | 1.5 | 1.3 | 12.7 | 0.0004 | 1.225 |
| 5. Number of repetitions of third syllable | 0.1 | 0.4 | 0.2 | 0.6 | 0.1 | 0.70 | −0.216 |
| 6. Song duration (sec) | 1.5 | 0.2 | 1.3 | 0.2 | 11.0 | 0.001 | 0.176 |
| 7. Duration of part I (sec) | 1.0 | 0.2 | 1.0 | 0.3 | 1.0 | 0.32 | 0.443 |
| 8. Duration of part II (sec) | 0.5 | 0.2 | 0.2 | 0.2 | 13.3 | 0.0003 | −0.086 |
| 9. Minimum frequency (Hz) | 2326.2 | 555.9 | 2172.2 | 1096.8 | 7.2 | 0.007 | 0.701 |
| 10. Maximum frequency (Hz) | 7617.3 | 964.6 | 6395.9 | 1340.9 | 12.6 | 0.0004 | 0.948 |
| 11. Number of notes of the last syllable from part I | 4.6 | 1.0 | 3.6 | 1.3 | 8.6 | 0.003 | 0.313 |
| 12. Number of notes of the first syllable from part II | 4.0 | 2.3 | 1.5 | 1.3 | 18.7 | <0.0001 | 0.249 |
| 13. Maximum frequency of last syllable from part I (Hz) | 7146.6 | 1148.7 | 6335.8 | 837.3 | 7.2 | 0.007 | −0.446 |
| 14. Minimum frequency of last syllable from part I (Hz) | 2989.2 | 570.2 | 2526.1 | 672.6 | 5.6 | 0.02 | 0.205 |

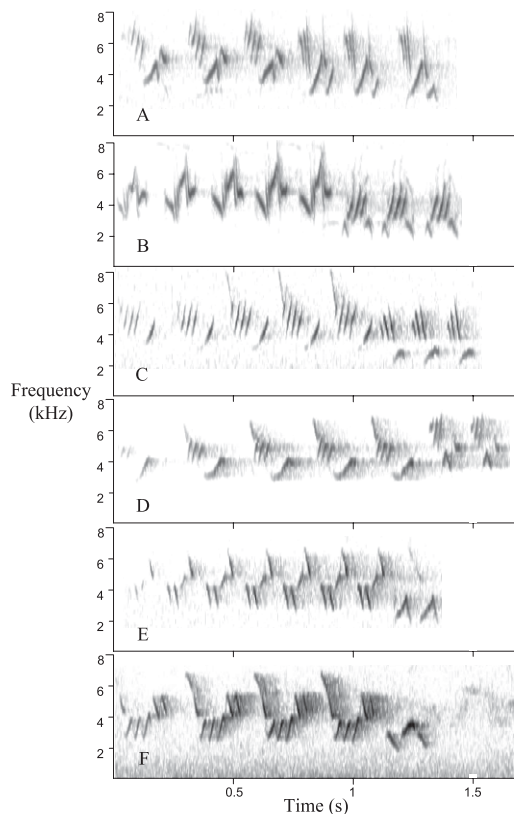


FIGURE 3. Song spectrograms produced in RAVEN for MacGillivray's and Mourning Warblers recorded across the breeding ranges. Recordings from Prince George, British Columbia (A), and Kernville, California (B), represent allopatric MacGillivray's Warblers. Recordings from Tumbler Ridge, British Columbia (C), and Pine Pass, British Columbia (D), represent songs from the hybrid zone. Recordings from Lesser Slave Lake, Alberta (E), and Sturgeon Falls, Ontario (F), represent allopatric Mourning Warblers.

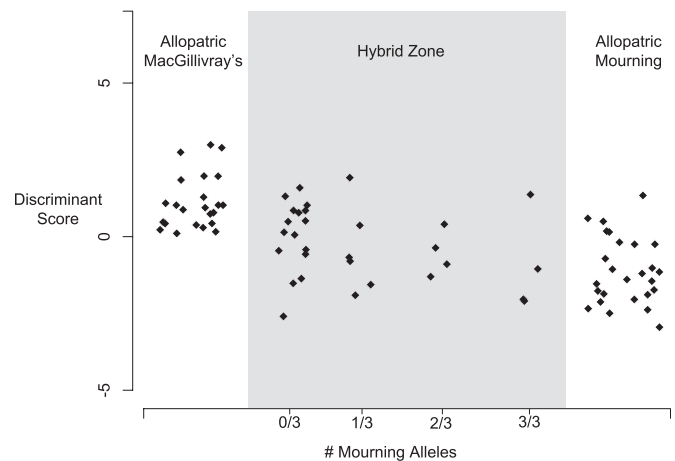


FIGURE 4. Discriminant scores and molecular hybrid index of MacGillivray's, Mourning and hybrid MacGillivray's \times Mourning Warblers in allopatry ($n = 49$) and sympatry ($n = 30$). The discriminant function was based on 14 song variables from individuals outside of the hybrid zone. Individuals in the hybrid zone are categorized by the number of Mourning Warbler alleles from a diagnostic mitochondrial (COI) and a Z-linked (CHD1Z) marker (i.e., a pure male Mourning Warbler has the mitochondrial allele and both CHD1Z alleles typical of the Mourning Warbler). There is a weak and nonsignificant association between song type and genetic identity in the hybrid zone (correlation coefficient: $r_s = -0.32$, $P = 0.08$).

as MacGillivray's song = 0.84) and Mourning Warblers (average probability of assignment as MacGillivray's song = 0.21). There was a weak and nonsignificant association within the hybrid zone between genotype (as determined by the bird's number of eastern alleles) and song as characterized by the DFA according to the classification scores (correlation coefficient: $r_s = -0.32$, $P = 0.08$) or binary posterior assignment ($z = -1.6$, $P = 0.09$). We observed no association between

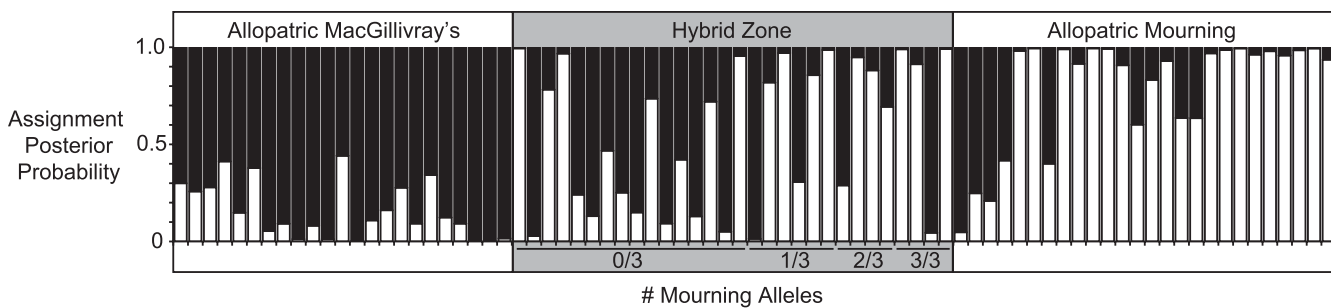


FIGURE 5. Posterior probability of individuals assigned as either a MacGillivray's (black) or Mourning Warbler (white) singer from the discriminant function analysis. Each column represents a single individual with the proportion of black or white representing the probability of the DFA assigning its song as MacGillivray's-like or Mourning-like, respectively. Individuals in the hybrid zone are distinguished by their number of Mourning Warbler alleles (see Figure 3). There is a weak and nonsignificant association between the song-type assignment and genotype in the hybrid zone ($z = -1.64$, $P = 0.09$).

morphological characters and song within the hybrid zone; there was no significant association between song as characterized by the DFA and eye-arc score ($r_s = -0.2025$, $P = 0.2832$) or wing-tail ($r_s = 0.0344$, $P = 0.8566$).

DISCUSSION

The recent finding of extensive hybridization between MacGillivray's and Mourning Warblers (Irwin et al. 2009) was surprising, given that these two forms have been considered distinct species since their original description and have divergent songs in areas of allopatry (Pitocchelli 1990). While this finding might imply a lack of any reproductive isolation between these forms, the narrowness of the hybrid zone relative to the assumed distance of these birds' dispersal suggests that some form of selection maintains the zone (Irwin et al. 2009). The goal of this subsequent study was to determine whether song is an important reproductive barrier between these species in the area where they hybridize. To do this we first used a combination of song characteristics to distinguish between songs of allopatric MacGillivray's and Mourning Warblers, then asked how these characteristics varied across the hybrid zone and whether song is correlated with genotype.

Our analysis found significant differences in song between allopatric MacGillivray's and Mourning Warblers, as has been noted previously (Pitocchelli 1990). For instance, in a principal components analysis of the two species' songs Pitocchelli (1990) found that a song's number of syllable types had the highest loading on PC1 (explaining 44% of variation). We also found that the taxa differ significantly in this variable, although our DFA found that the number of repetitions of the second syllable was best at distinguishing them. In contrast to the situation of the Pacific and Winter Wrens, distinguished by a few variables (i.e., mean frequency, high frequency, and the rate of transitions in sound frequency from below to above 5.5 kHz) in a single song (Toews and Irwin 2008), evidently no single song character distinguishes MacGillivray's and Mourning Warbler songs clearly.

Using a multivariate DFA, however, we were able to classify individuals in allopatry confidently as either a MacGillivray's or Mourning singer between 73 and 90% of the time, depending on the cross-validation method we used. The three groups (MacGillivray's, Mourning, and hybrid-zone warblers) differed significantly in their discriminant scores. Individuals in the hybrid zone, on average, had songs that tended to have discriminant scores intermediate between recordings from farther east and west, but because these songs all fall within the range of natural variation observed in allopatric MacGillivray's and Mourning Warblers, this intermediacy does not suggest the presence of a novel hybrid song type.

Interestingly, in spite of the expectation that nonrandom associations between genetic markers and traits should be observed in a tension zone (Barton and Hewitt 1985), we found no significant relationship in the hybrid zone between the song that a bird sings and species-diagnostic mitochondrial and nuclear genetic markers. This observation is in agreement with our qualitative observations of song in the hybrid zone: song is not predictive of a bird's plumage or morphological characteristics, which are correlated with genetic assignment (Irwin et al. 2009; Mark Phinney, pers. comm.). One reason that we might not expect an association between song type and genotype in the hybrid zone is that birds may learn songs from both conspecifics and heterospecifics indiscriminately. Many passerines, including other wood-warblers closely related to *Oporornis*, learn their songs in their first year from male tutors in neighboring territories (Spector 1992, Catchpole and Slater 1995, Forstmeier 2009). Indeed, recent studies of interspecific song learning between divergent species of wood warblers illustrate the possibility for both intermediate song (Vallender et al. 2009) or highly accurate copying (Boves et al. 2010). If sexual selection on song is strong, then singing the incorrect song in the hybrid zone could have consequences for fitness (Clayton 1990, Baker and Baker 1990). If, however, there is little or no fitness cost to a genetically Mourning-like individual learning a MacGillivray's-like song

(and *visa versa*), then we might expect cultural transmission and introgression of song between the species to occur.

The lack of striking song differences between these taxa in allopatry in combination with indiscriminate song learning in the contact zone could contribute to the continued hybridization observed by Irwin et al. (2009). A study of another oscine species pair in Europe suggests a similar scenario: Haavie et al. (2004) identified heterospecific song learning as a potential proximate mechanism explaining hybridization between the Pied (*Ficedula hypoleuca*) and Collared (*F. albicollis*) Flycatchers.

Alternatively, song convergence in sympatry could be due to ecological factors, such as signal constraints imposed by the acoustic environment (Secondi et al. 2003). Coincident with the genetic transition between these species is also a subtle morphological transition between them, the Mourning Warbler having a larger difference in length between the tail and the wing (Irwin et al. 2009). Indeed, the fact that the center of this hybrid zone is within the broad transitional area between the mixed-wood boreal forest east of the Rockies and the montane, conifer-dominated forests to the west is also consistent with a habitat-dependent model. This possibility should be treated with caution, however, as the ecological relevance of the subtle morphological differences between these taxa is debatable. In addition, although a detailed study of habitat differences between this and other avian pairs that come into contact in this area is lacking, MacGillivray's and Mourning Warblers share generally similar habitat preferences, both favoring mixed deciduous habitat and with moderate cover and dense undergrowth (Pitocchelli 1993, 1995, Irwin et al. 2009).

Ideally, to address the question of pre-mating isolation and assortative mating fully, an analysis such as this should be paired with playback experiments to estimate potential variation in male and female responses to different songs. Pitocchelli (1990) referred to preliminary data suggesting that the two species can distinguish between the different songs in allopatry (Pitocchelli 1990), but further work is needed to confirm this finding and to test song recognition in the hybrid zone and areas of allopatry. Den Hartog et al. (2007) found that in a hybrid zone the doves *Streptopelia vinacea* and *S. capicola* did not differ in their responses to songs of hybrid or allopatric birds but that individuals from allopatric populations responded to hybrids' song to a degree intermediate between their responses to conspecific and heterospecific songs. They suggested that this may reduce the ability of hybrid singers to hold a territory outside of the hybrid zone. We suggest that similar playback experiments be conducted on the Mourning and MacGillivray's Warblers to determine whether songs of individuals in the hybrid zone also produce a strong response in populations far to the west and east. In addition, an analysis of call notes may reveal a stronger association with genetic patterns than we found with song—Irwin et al. (2008) observed such a pattern in the Greenish Warbler (*Phylloscopus trochiloides*)—but observations in the field suggest that the call notes of MacGillivray's and Mourning

Warblers in the contact zone also overlap (Mark Phinney, pers. comm.), although there has been no quantitative analysis of these calls to date.

The result of our study is interesting in the context of other west–east counterpart species that also come into contact in northeastern British Columbia. For instance, in their study of a contact zone between the Pacific and Winter Wrens Toews and Irwin (2008) found that these taxa maintain striking song differences in sympatry, despite an apparent absence of morphological differences. One reason why these two species may have evolved sufficient pre-mating reproductive barriers is that their estimated time of divergence (approximately 4.3 million years before present; Toews and Irwin 2008) is much older than that between MacGillivray's and Mourning Warblers (1.1 million years before present; Weir and Schluter, 2004), giving them more time to accumulate such differences.

Our finding of little evidence of pre-mating isolation in the form of song suggests another form of selection, post-mating selection against hybrids, may play an important role in maintaining this narrow hybrid zone. While previous studies have found that full post-mating isolation can take tens of millions of years to develop in birds, at least partial reproductive isolation between taxa can arise within only a few million years (Price and Bouvier 2002). This is highlighted by recent study of the hybrid zone between the Collared and Pied flycatchers, where there is evidence of selection against hybrids after less than 1 million years of independent evolution (reviewed in Qvarnström et al. 2010). In another example, Audubon's and Myrtle Warblers, which also hybridize along the Rockies, show little evidence of assortative mating based on plumage, and while no detailed analysis of their song has been published, differences between the songs of the two taxa are slight, suggesting a potentially important role for post-mating isolation (Brelsford and Irwin 2009). Forms of post-mating isolation that could be involved in such cases include divergent habitat preference or migratory behavior, producing suboptimal characteristics in hybrids (Coyne and Orr 2004, Irwin and Irwin 2005). We suggest that habitat-based post-mating isolation in the case of MacGillivray's and Mourning Warblers is unlikely, as both taxa seem to prefer mixed deciduous habitat with dense undergrowth and moderate cover (Pitocchelli 1990, 1993, 1995, Irwin et al. 2009), and both can be found in similar habitats within the contact zone. Divergent migratory routes, however, may play an important role here, as these taxa differ in their migratory routes and winter ranges (Pitocchelli 1993, 1995).

In conclusion, we find that while in allopatry there are differences in song between MacGillivray's and Mourning Warblers, these differences are greatly reduced in sympatry, where there is no significant correlation between song and genotype. This finding is in contrast with a number of other cases of diverging avian taxa in which song appears to be an important barrier to reproduction (Päckert and Martens 2004, Irwin et al. 2008, Toews and Irwin 2008). We suggest

that heterospecific song learning could be contributing to hybridization between these two species. Study of this and other hybrid zones between closely related taxa in secondary contact will allow us to continue to address fundamental questions about how selection acts in nature and which genes and traits may be involved in the evolution of reproductive isolation.

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LITERATURE CITED

- BAKER, M. C., AND E. M. BAKER. 1990. Reproductive behavior of female buntings: isolating mechanisms in a hybridizing pair of species. *Evolution* 44:332–338.
- BARTON, N. H. 1983. Multilocus clines. *Evolution* 37:454–471.
- BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16:113–48.
- BOVES, T. J., BUEHLER, D. A., AND P. C. MASSEY. 2010. Interspecific song imitation by a Cerulean Warbler. *Wilson Journal of Ornithology* 122:583–587.
- BRELSFORD, A., AND D. E. IRWIN. 2009. Incipient speciation despite little assortative mating: the Yellow-rumped Warbler hybrid zone. *Evolution* 63:3050–3060.
- CATCHPOLE, C. K., AND P. J. B. SLATER. 1995. *Bird song: biological themes and variations*. Cambridge University Press, Cambridge, England.
- CLAYTON, N. S. 1990. Assortative mating in Zebra Finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanotis*. *Philosophical Transactions of the Royal Society of London B* 1258:351–370.
- COYNE, J. A., AND H. A. ORR. 2004. *Speciation*. Sinauer Associates, Sunderland, MA.
- DALZIEL, A. C., S. M. ROGERS, AND P. M. SCHULTE. 2009. Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. *Molecular Ecology* 18:4997–5017.
- DE KORT, S. R., P. M. DEN HARTOG, AND C. TEN CATE. 2002. Diverge or merge? The effect of sympatric occurrence on the territorial vocalizations of the Vinaceous Dove *Streptopelia vinacea* and the Ring-necked Dove *S. capicola*. *Journal of Avian Biology* 33:150–158.
- DEN HARTOG, P. M., S. R. DE KORT AND C. TEN CATE. 2007. Hybrid vocalizations are effective within, but not outside, an avian hybrid zone. *Behavioral Ecology* 18:608–614.
- FORSTMEIER, W., C. BURGER, K. TEMNOW, AND S. DERÉGNAUCOURT. 2009. The genetic basis of Zebra Finch vocalizations. *Evolution* 63:2114–2130.
- GEE, J. M. 2005. No species barrier by call in an avian hybrid zone between California and Gambel's Quail (*Callipepla californica* and *C. gambelii*). *Biological Journal of the Linnean Society* 86:253–264.
- HAAVIE, J., T. BORGES, S. BURES, L. Z. GARAMSZEGI, H. M. LAMPE, J. MORENO, A. QVARNSTRÖM, J. TÖRÖK, AND G. SÆTRE. 2004. Flycatcher song in allopatry and sympatry—convergence, divergence and reinforcement. *Journal of Evolutionary Biology* 17:227–237.
- IRWIN, D. E. 2000. Song variation in an avian ring species. *Evolution* 54:998–1010.
- IRWIN, D. E., AND J. H. IRWIN. 2005. Siberian migratory divides: the role of seasonal migration in speciation. *In* R. Greenberg and P. P. Marra [EDS.], *Birds of two worlds: the ecology and evolution of migration*. Johns Hopkins University Press, Baltimore.
- IRWIN, D. E., M. P. THIMGAN, AND J. H. IRWIN. 2008. Call divergence is correlated with geographic and genetic distance in Greenish Warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology* 21:435–448.
- IRWIN, D. E., A. BRELSFORD, D. P. L. TOEWS, C. MACDONALD, AND M. PHINNEY. 2009. Extensive hybridization in a contact zone between MacGillivray's Warblers (*Oporornis tolmiei*) and Mourning Warblers (*O. philadelphia*) detected using molecular and morphological analyses. *Journal of Avian Biology* 40:539–552.
- LACHLAN, R. F., AND M. R. SERVEDIO. 2004. Song learning accelerates allopatric speciation. *Evolution* 58:2049–2063.
- MENGEL, R. M. 1964. The probable history of species formation in some northern wood warblers (Parulidae). *Living Bird* 3:9–43.
- PÄCKERT, M., AND J. MARTENS. 2004. Song dialects on the Atlantic islands: Goldcrests of the Azores (*Regulus regulus azoricus*, *R. r. sanctae-mariae*, *R. r. inermis*). *Journal of Ornithology* 145:23–30.
- PITOCHELLI, J. 1990. Plumage, morphometric and song variation in the Mourning (*Oporornis philadelphia*) and MacGillivray's (*O. tolmiei*) Warblers. *Auk* 107:161–171.
- PITOCHELLI, J. 1992. Plumage and size variation in the Mourning Warbler. *Condor* 94:198–209.
- PITOCHELLI, J. 1993. Mourning Warbler (*Oporornis philadelphia*), no. 72. *In* A. Poole and F. Gill [EDS.], *The birds of North America*. Academy of Natural Sciences, Philadelphia.
- PITOCHELLI, J. 1995. MacGillivray's Warbler (*Oporornis tolmiei*), no. 159. *In* A. Poole and F. Gill [EDS.], *The birds of North America*. Academy of Natural Sciences, Philadelphia.
- PRICE, T. 2008. *Speciation in birds*. Roberts and Company, Greenwood Village, CO.
- PRICE, T. D., AND M. M. BOUVIER. 2002. The evolution of F1 postzygotic incompatibilities in birds. *Evolution* 56:2083–2089.
- QVARNSTRÖM, A., A. M. RICE, AND H. ELLEGREN. 2010. Speciation in *Ficedula* flycatchers. *Philosophical Transactions of the Royal Society B* 365:1841–1852.
- RIESEBERG, L. H., AND C. A. BUERKLE. 2002. Genetic mapping in hybrid zones. *American Naturalist* 159:S36–S50.
- ROBBINS, M. B., M. J. BRAUN, AND E. A. TOBEY. 1986. Morphological and vocal variation across a contact zone between the chickadees *Parus atricapillus* and *P. carolinensis*. *Auk* 103:655–666.
- ROHWER, S., AND C. WOOD. 1998. Three hybrid zones between Hermit and Townsend's Warblers in Washington and Oregon. *Auk* 115:284–310.
- SÆTRE, G.-P., T. MOUM, S. BUREŠ, M. KRÁL, M. ADAMJAN, AND J. MORENO. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387:589–592.
- SECONDI, J., V. BRETAGNOLLE, C. COMPAGNON, AND B. FAIVRE. 2003. Species-specific song convergence in a moving hybrid zone between two passerines. *Biological Journal of the Linnean Society* 80:507–517.

- SLABBEKOORN, H., AND T. B. SMITH. 2002. Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society of London B* 357:493–503.
- SPECTOR, D. A. 1992. Wood-warbler song systems: a review of paruline singing behaviors. *Current Ornithology* 9:199–238.
- TOBIAS, J. A., AND N. SEDDON. 2009. Signal design and perception in *Hypocnemis* antbirds: evidence for convergent evolution via social selection. *Evolution* 63:3168–3189.
- TOEWS, D. P. L., A. BRELSFORD, AND D. E. IRWIN. In press. Hybridization between Townsend's and Black-throated Green Warblers in an avian suture zone. *Journal of Avian Biology*.
- TOEWS, D. P. L., AND D. E. IRWIN. 2008. Cryptic speciation in a holarctic passerine revealed by genetic and bioacoustic analyses. *Molecular Ecology* 17:2691–2705.
- VALLENDER, R., J.-P. GAGNON, AND I. LOVETTE. 2009. An intergeneric wood-warbler hybrid (*Mniotilta varia* × *Dendroica coronata*) and use of multilocus DNA analyses to diagnose avian hybrid origins. *Wilson Journal of Ornithology* 121:298–305.
- WEIR, J. T., AND D. SCHLUTER. 2004. Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society of London B* 271:1881–1887.

APPENDIX. Here we present a discriminant analysis similar to that described above but based on only those birds that sang a song of two syllables ($n = 65$, 42 from allopatric populations and 23 from the hybrid zone). By the classification function derived from the discriminant analysis of all of the allopatric individuals the misclassification rate was 7%. When we used the leave-one-out method of sequential removal of individual data points the misclassification rate rose to 43%.

The discriminant scores of the groups differed significantly (MacGillivray's, Mourning, and hybrid zone; $\chi^2 = 25.9$, $df = 2$, $P = <0.0001$; Kruskal–Wallis nonparametric test). In a post hoc analysis, there was a significant difference in the discriminant scores between two of the three groups: MacGillivray's vs. Mourning ($U = 24.9$, $df = 1$, $P = <0.0001$) and Mourning vs. hybrid zone ($U = 9.9$, $df = 1$, $P = 0.02$). The difference MacGillivray's vs. hybrid zone was not significant by the criterion of our conservative α of 0.017 ($U = 4.5$, $df = 1$, $P = 0.03$).

Again, using only birds with a two-syllable song we found no evidence of the existence of a novel song type in the hybrid zone. There was a weak and nonsignificant association between genotype (as determined by a bird's number of eastern alleles) and song type as characterized by the discriminant function analysis using the classification scores (correlation coefficient: $r_s = -0.36$, $P = 0.09$) or binary posterior assignment ($z = -1.37$, $P = 0.17$). There was no association between morphological characters and song within the hybrid zone; there was no significant association between song as characterized by the discriminant function analysis and eye-arc score ($r_s = -0.2791$, $P = 0.1971$) or the wing – tail measurement ($r_s = -0.2031$, $P = 0.3528$).

Means, standard deviations, Mann–Whitney U -tests and factor loadings from discriminant function analysis for 16 song variables of allopatric MacGillivray's ($n = 23$) and Mourning ($n = 19$) Warblers based on only those individuals which have a second syllable type in their song.

| Variable | Allopatric MacGillivray's Warblers | | Allopatric Mourning Warblers | | Mann–Whitney U -test ($df = 1$) | | DFA factor loadings |
|---|------------------------------------|--------|------------------------------|--------|-------------------------------------|-------|---------------------|
| | Mean | SD | Mean | SD | U | P | |
| 1. Number of syllable types | 2.1 | 0.3 | 2.0 | 0.3 | 0.1 | 0.78 | 0.559 |
| 2. Total number of syllables per song | 7.3 | 1.0 | 7.0 | 1.3 | 0.6 | 0.43 | –0.721 |
| 3. Number of repeats of first syllable | 4.6 | 0.7 | 4.7 | 1.3 | 0.2 | 0.69 | 0.134 |
| 4. Number of repeats of second syllable | 2.7 | 0.9 | 2.0 | 1.1 | 6.0 | 0.01 | 1.257 |
| 5. Number of repeats of third syllable | 0.1 | 0.4 | 0.3 | 0.7 | 0.6 | 0.45 | 0.046 |
| 6. Song duration (sec) | 1.5 | 0.2 | 1.4 | 0.2 | 8.2 | 0.004 | –0.496 |
| 7. Duration of part I (sec) | 1.0 | 0.2 | 0.9 | 0.2 | 0.0002 | 0.99 | 1.032 |
| 8. Duration of part II (sec) | 0.5 | 0.2 | 0.3 | 0.2 | 6.6 | 0.01 | 0.149 |
| 9. Minimum frequency (Hz) | 2326.2 | 555.9 | 1890.7 | 311.6 | 9.4 | 0.002 | 0.852 |
| 10. Maximum frequency (Hz) | 7617.3 | 964.6 | 6876.7 | 831.7 | 6.2 | 0.01 | 0.406 |
| 11. Number of notes of the last syllable from part I | 4.6 | 1.0 | 3.6 | 1.4 | 5.2 | 0.02 | 0.212 |
| 12. Number of notes of the first syllable from part II | 4.0 | 2.3 | 2.0 | 1.2 | 11.6 | 0.001 | 0.457 |
| 13. Maximum frequency of last syllable from part I (Hz) | 7146.6 | 1148.7 | 6467.2 | 919.9 | 3.9 | 0.05 | –0.164 |
| 14. Minimum frequency of last syllable from part I (Hz) | 2989.2 | 570.2 | 2757.1 | 619.5 | 1.2 | 0.27 | –0.076 |
| 15. Maximum frequency of first syllable from part II (Hz) | 6769.7 | 1425.4 | 5053.7 | 1473.1 | 10.7 | 0.001 | 0.122 |
| 16. Minimum frequency of first syllable from part II (Hz) | 2417.9 | 469.6 | 2149.0 | 477.9 | 2.8 | 0.09 | –0.250 |