

MALE SONG VARIATION AND FEMALE MATE CHOICE IN THE GOLDEN-WINGED WARBLER

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Abstract. We investigated the distinctiveness of males' song and the relationship between song variables and females' mate choice in the Golden-winged Warbler (*Vermivora chrysoptera*). We recorded the songs of 23 males and quantified them to examine performance-related traits and specific song content. We carried out discriminant function analysis to compare variation within and among males for characteristics of both song types I and II. We used microsatellite DNA to identify the parents of 62 nestlings from 17 nests. By assigning paternity to males in our population we could examine relationships between females' choice and variation in males' song by comparing characteristics of within- and extra-pair sires. While we were able to discriminate statistically between individual males' songs, we found no significant differences in song variables between the within- and extra-pair males at a given nest. Therefore, female Golden-winged Warblers do not appear to be using these differences in song in choosing a mate. Further research on the Golden-winged Warbler is needed to understand song function and to gain insight into this species' reproductive biology, both of which could have important conservation implications for this declining species.

Key words: *Golden-winged Warbler, mate choice, paternity, song, Vermivora chrysoptera.*

Variación del Canto en Machos y Selección de Pareja por parte de la Hembra en *Vermivora chrysoptera*

Resumen. Investigamos los rasgos distintivos de los cantos de los machos y la relación entre las variables de los cantos y la selección de pareja por parte de las hembras en *Vermivora chrysoptera*. Registramos y cuantificamos los cantos de 23 machos para examinar los rasgos vinculados con el desempeño y los contenidos específicos de cada canto. Realizamos análisis de función discriminante para comparar la variación en un mismo macho y entre machos de las características de los canto tipo I y II. Empleamos ADN microsatelital para identificar a los padres de 62 pichones provenientes de 17 nidos. Mediante la asignación de paternidad a los machos de nuestra población, pudimos examinar la relación entre la elección de las hembras y las variaciones en los cantos de los machos comparando las características de los progenitores de una pareja y los externos a las parejas. A pesar de que fuimos capaces de discriminar estadísticamente entre los cantos individuales de los machos, no encontramos diferencias significativas en las variables de los cantos entre los machos de una pareja y los externos a las parejas para un nido determinado. Por ende, las hembras de *V. chrysoptera* no parecen estar usando estas diferencias en los cantos para elegir a su pareja. Se necesita más investigación sobre *V. chrysoptera* para entender la función del canto y para mejorar los conocimientos de la biología reproductiva de la especie. Ambas líneas de investigación pueden tener implicancias importantes para la conservación de esta especie cuyas poblaciones están disminuyendo.

INTRODUCTION

SONG DISTINCTIVENESS AND MATE CHOICE

Among the songbirds, song is an important variable in species recognition (e.g., Marler and Peters 1977, Mathevon and Aubin 2001), territorial defense (e.g., Searcy et al. 2000, Trillo and Vehrencamp 2005), and mate attraction (e.g., Hasselquist et al. 1996, Nowicki et al. 1998). The latter two functions are frequently studied because they relate directly to reproductive success via mate choice and male–male competition, thus providing insight into the adaptive significance of song variation (reviewed by Gil and Gahr 2002).

Birds also use song to identify conspecific neighbors (Weary et al. 1987) and to distinguish individuals (Lind et al. 1996, Sharp and Hatchwell 2005, Wisniewski and Hulse 1997). The ability of females to use song to distinguish among males can play an important role in decisions about pairing and mate choice and thus in sexual selection. Indirect empirical studies have positively correlated bird song with indicators of fitness such as arrival date (Arvidsson and Neergaard 1991), territory quality (Arvidsson and Neergaard 1991, Gottlander 1987, Radesater et al. 1987, Hoi-Leitner et al. 1995), early pairing (Alatalo et al. 1990), timing of female fertility

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(Mace 1987), and decreased cuckoldry (Møller et al. 1998). These relationships suggest that females are assessing males on the basis of particular song traits and are using these cues to inform their mate-choice decisions.

Past research on this topic has focused primarily on the degree of elaboration of bird song (e.g., repertoire size) because it is assumed to have evolved via sexual selection, song variables being correlated with an individual's quality. For example, complex song repertoires provide an honest signal of high male quality (Hasselquist et al. 1996). Few studies have investigated the role of specific fine-scale song traits (e.g., frequency and trill rates) in mate choice (Byers 2007), yet females may use such factors when choosing both within-pair and extra-pair partners. These traits may be especially pertinent in species in which repertoire size does not vary. Examining song characteristics in species with small repertoires enables us to examine the function of fine-scale traits related to females' mate choice, independently from song diversity (Byers 2007). The Golden-winged Warbler (*Vermivora chrysoptera*) has a small repertoire and tends to sing songs that are context specific (Morse 1989). Therefore, it is a species appropriate for investigating the role of fine-scale song traits in mate choice.

STUDY SPECIES' SONG

The Golden-winged Warbler is a socially monogamous neotropical migrant that, like the other Parulidae, sings two categories of songs: song type I for mate attraction and species recognition, type II for territorial defense and during aggressive interactions between males (Spector 1992). Song type I has two syllable types and is generally described as *zee-bee-bee-bee*; the first syllable is a buzzy high-frequency phrase, followed by one to six (usually three) buzzy phrases at a lower frequency (Highsmith 1989a; Fig. 1). This song type is typically sung in the upper quarter of the canopy, seemingly to enhance the male's vocal and visual display (Highsmith 1989a, Rossell 2001). The first syllable of song type I has been suggested to serve as a species identifier since playback experiments showed that a *zee* syllable must precede a *bee* syllable in order to elicit a response within the species (Ficken and Ficken 1973). This feature is of interest because some individuals have a monotone type I song in which there is such little difference in the *zee* and *bee* phrases and so might be considered to lack the *zee* phrase entirely (Highsmith 1989a; S. Harper, pers. obs.). Highsmith (1989a) noted two different categories of type I song: long type I, which is sung before pairing, and short type I, which is sung after pairing.

The Golden-winged Warbler's song type II has three to five syllable types and is typically sung at the edge of the male's territory likely as a means of territorial advertisement and defense. It is also used during aggressive interactions between males, and it is this song type that is sung during the dawn chorus (Highsmith 1989a, Fig. 1). Therefore, type II

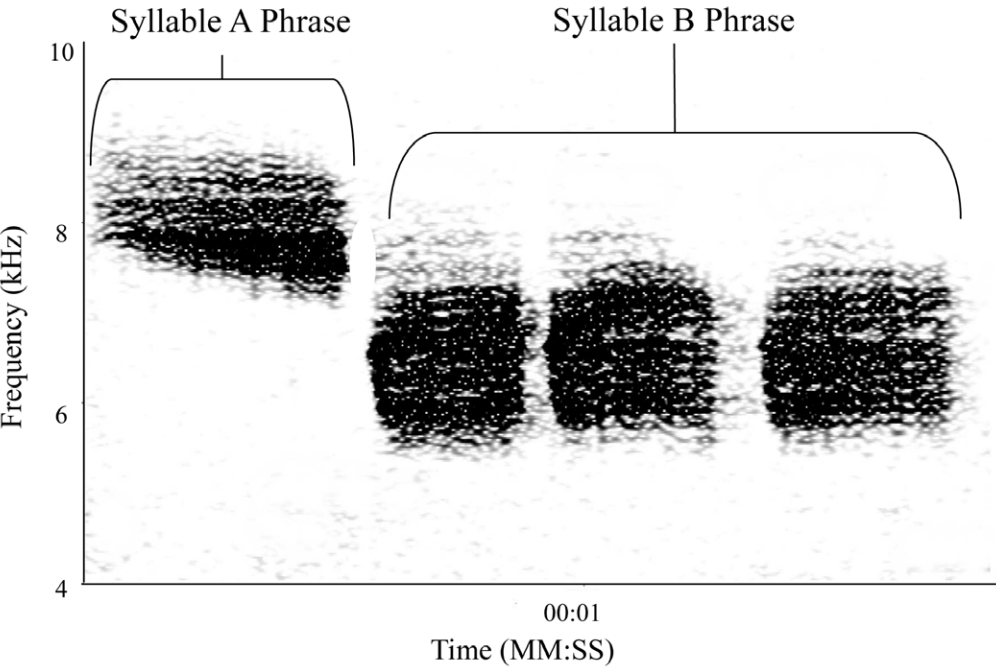
song is thought to be primarily an intrasexual signal, defining an individual's territory and warding off intruders. However, it is also possible that females are listening to male-male song competition and potentially assessing males by this cue (Mennill et al. 2002). Indeed, Mennill et al. (2002) found that a song can function as both mate attraction and territory defense simultaneously.

BREEDING BIOLOGY

Golden-winged Warblers are known to produce broods of mixed paternity via extra-pair fertilizations (Reed et al. 2007, Vallender et al. 2007a). Moreover, Golden-winged Warblers regularly hybridize and produce fertile offspring with the congeneric Blue-winged Warbler (*V. pinus*; Gill 1980, Neville et al. 2008). Hybrids sing song type I of either the Golden-winged or the Blue-winged Warbler, and both species have been known to sing the other's song type I (Ficken and Ficken 1968; R. Vallender, pers. obs.). Rarely, phenotypic Blue-winged and Golden-winged Warblers have a bivalent repertoire and sing both species' songs (Rossell 2001; R. Vallender, pers. obs.). Song type II, however, is virtually identical in the Golden-winged Warbler, Blue-winged Warbler, and their hybrids, although regional dialects are known (Highsmith 1989b). Similarities in song between closely related species has been hypothesized to be one cause of hybridization (i.e., leading to mate-recognition errors; Randler 2002), and that may be one reason for the widespread hybridization between the Golden-winged and Blue-winged Warblers (Vallender et al. 2007b). At the least, an overlap in song elements may complicate females' choosing conspecific mates (Randler 2002).

Currently, the Golden-winged Warbler is one of the fastest declining passerines in North America (Sauer et al. 2008). The overall population decline is approximately 3% yr⁻¹, with regional declines as high as 31% year⁻¹ (Sauer et al. 2008). The species has no federal protection in the United States but was recently listed as threatened in Canada by the Committee on the Status of Endangered Wildlife in Canada (www.sararegistry.gc.ca). To date, two studies using genetic analyses of parentage have examined the Golden-winged Warbler's reproductive success (Reed et al. 2007, Vallender et al. 2007a). Vallender et al. (2007a) compared a suite of phenotypic characteristics of within- and extra-pair males but found no evidence that females were selecting males on the basis of any obvious physical characteristics. Therefore, they suggested that females were basing their mate-choice decisions on other male characteristics, such as song. An improved understanding of the role of song in this species' reproductive biology may have important conservation implications considering that hybrids can sing either a Golden-winged or a Blue-winged Warbler mate-attraction song, the essentially shared song type II, and the observed (although infrequent) mismatch between song type and phenotype. Understanding the role of song in the Golden-winged Warbler's mate choice

A) Type I Song



B) Type II Song

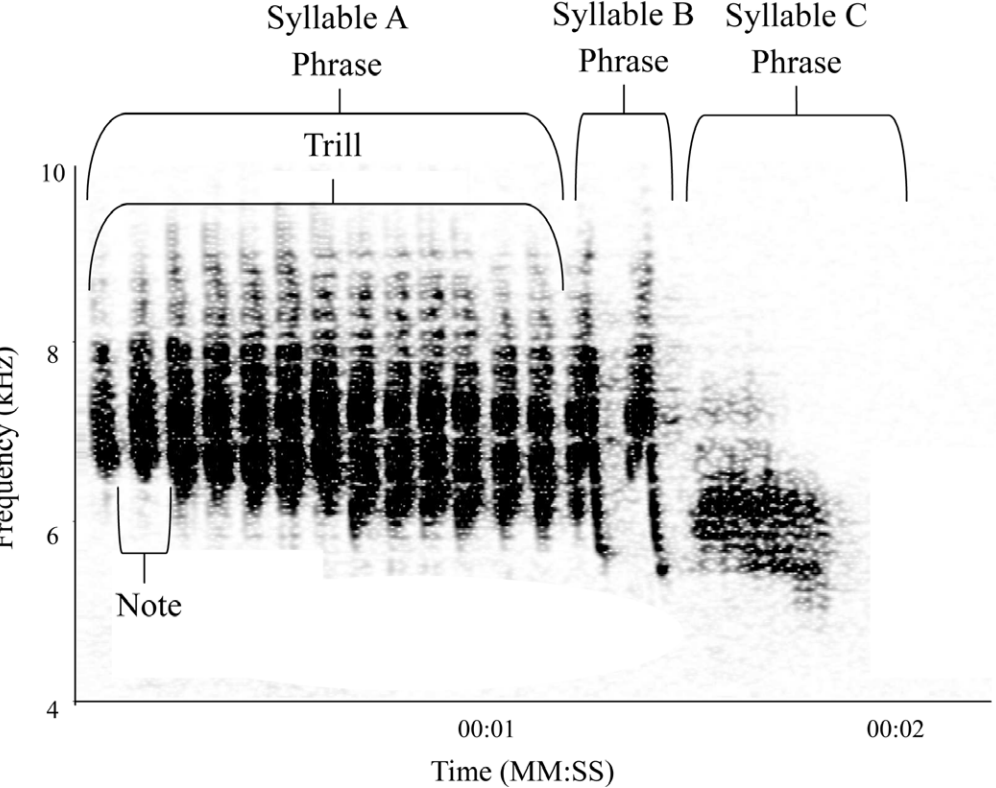


FIGURE 1. Sonograms of the Golden-winged Warbler’s type I (A) and type II (B) songs, indicating song structures.

may shed light on the largely unknown mechanisms of mate selection in this species.

STUDY GOALS

The goals of our study were to (1) determine whether the songs of male Golden-winged Warblers are individually distinct; and (2) examine the role of fine-scale song traits in female Golden-winged Warblers' mate choice. If males' songs are individually distinct, females might be using these fine-scale differences in song to assess males' quality. Thus, if females are choosing their extra-pair mates on the basis of song traits, we should expect the fine-scale song traits of her extra-pair mates to differ from those of her social mate.

METHODS

GENERAL FIELD METHODS

We conducted field work at the Queen's University Biological Station near Chaffey's Locks, Ontario (44° 34' N, 76° 19' W), from 28 April to 15 July 2005. The study population consisted predominantly of phenotypic Golden-winged Warblers; however, Blue-winged Warblers and hybrids have been observed in the area over the past 15 years (R. J. Robertson and T. Demmons, unpubl. data; Weir 1989), and the first Blue-winged Warbler nest was found in 2005 (R. Vallender, unpubl. data). Despite a short history of hybridization in this region, Vallender et al. (2007b) found that as many as 33% of phenotypic Golden-winged Warblers there are genetically introgressed, providing evidence for significant levels of cryptic hybridization. However, given that in this area the reproductive success of hybrids equals that of Golden-winged Warblers (Vallender et al. 2007a), and also because in songbirds song can be learned (Lachlan and Slater 1999), we did not take individual genetic status into account during our analyses.

We captured adult males by target mist netting using audio playback and a painted decoy, adult females by flushing them off the nest into a mist net after day 6 of incubation. In total, we captured, banded, and released 53 males and 23 females. All adults were banded with an aluminum Canadian Wildlife Service (CWS) leg band and a unique combination of plastic color leg bands to enable identification of individuals. We collected a small (~20 µL) blood sample from the brachial vein and measured length of the right tarsus (mm) and mass (g). We aged adults as second year (SY) or after second year (ASY) by rectrix shape (Pyle 1997). More precise ages were calculated for birds that had been captured and marked in previous years (R. Vallender, unpubl. data). Our inclusion of age and body condition into our analyses was done under the assumption that both these factors may be correlated with a male's song: older males are more experienced and may sing a higher-quality song (Hasselquist et al. 1996, Gil et al. 2001), and larger males may be of better quality and thus more able to invest in singing (Gil and Gahr 2002).

We located nests by observing females carrying nesting material or by actively searching in suitable habitat at three study sites. Once a nest was located, we observed nest development and progress every second day through fledging or depredation. On the fourth day after hatching, we banded nestlings with a CWS band and took blood samples (~15 µL) from the metatarsal vein.

SONG RECORDINGS

We recorded songs from a total of 23 adult males from 18 May to 26 June 2005 by using either (A) a digital recorder (Marantz PMD670 Professional Solid State Portable Recorder) with a directional microphone (Audio-technica AT815a) or (B) a pre-amplifier (Saul-Mineroff), a tape recorder (Marantz PMD-222), and a directional microphone (Audio-technica 815b). For songs recorded with the digital recorder, the sampling rate was 16 bit, 22 050 Hz, and in WAV format. While some species' song rate changes through the breeding season, Highsmith (1989a) did not find significant variability through the breeding season in the Golden-winged Warbler's song structure or in the song rate of song types I or II; therefore, we considered any influence of the date of recording to be negligible. For each male, song type II was recorded between 04:30 and 06:00 EST (i.e., the dawn chorus; mean of 31 min of recording), as was the first 15 min of song type I immediately following the dawn chorus. The time, date, location, location changes, and behavioral observations were noted.

SONG ANALYSES

Tape recordings were digitized at a sampling rate of 22 050 Hz with SYRINX-PC sound-analysis software (John Burt, www.syrinxpc.com). The frequency of the maximum amplitude (FMA) was determined using Adobe Audition (version 2.0). SYRINX was used for all other song analyses with a transform size of 1024, Blackman fast Fourier transformation, and bandwidth from 4 to 10 kHz.

To quantify the rate of type I and type II songs, we followed methods described by Mennill and Vehrencamp (2005), using a fixed time display of 3 msec per line. We then analyzed the fine structure, using a fixed time display of 1 msec per line. To represent each individual male's type I and type II song, we sampled 10 songs of each type randomly from the male's entire recording. To do this, we used systematic random sampling, dividing recordings of each song type into a list of 10 consecutive segments, then randomly selecting a starting point and sampling at that same point in every segment. If a selected recording was of poor quality and could not be analyzed, then the next song was selected.

We defined syllables as discrete units separated by time on a sonogram and defined syllable types as described by Highsmith (1989a). For each type I and type II song sampled, we measured the FMA (kHz), maximum frequency (kHz),

minimum frequency (kHz), and duration (sec) of each phrase. We determined the trill rate for phrases A and B of song type II by dividing the number of notes by the length of the phrase to calculate notes per second (Hz).

PATERNITY ANALYSES

Full details of paternity methods can be found in Vallender et al. (2007a) and Reed et al. (2007). Briefly, polymerase chain reactions employed microsatellite loci isolated from the genomes of the Golden-winged Warbler (VeCr4, VeCr7, VeCr8; Stenzler et al. 2004) and Swainson's Warbler (*Limnothlypis swainsonii*; Lswul8; Winker et al. 1999).

The paternity analyses followed a three-step process detailed in Vallender et al. (2007a), using CERVUS v2.0 (available at <http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html>; Marshall et al. 1998), NewPat (available at <http://www.soo.xam.ac.uk/zoostaff/amos/newpat.htm>; Amos 2000), and all parental assignments were checked by hand.

STATISTICAL ANALYSES

Whenever possible, we aged birds as in their second, third, or fourth year or older by combining visual inspection (Pyle 1997) and retrospective data (R. Vallender, unpubl. data). We calculated seasonally corrected body mass by regressing weight over Julian capture date and saved the residuals. We then regressed these residuals over tarsus length and considered these residuals to indicate overall body condition.

To examine song variability, we followed the methods described by Lein (2008). Briefly, to calculate the magnitude of variation, we calculated the coefficients of variation (CV) for each variable for both song types. To measure the variation within a single recording, we calculated coefficients of variation of individual males, and coefficients of variation among males were calculated from variable means from each male. Then, to compare variation within and among males, we used univariable Welch's ANOVAs for each song variable.

Next, we attempted to statistically reduce the large number of song variables to a smaller number of principal components while keeping as much of the variation of the original characteristic as possible by conducting a principal component analysis (PCA). Then, we calculated a Kaiser–Meyer–Olkin measure of sampling adequacy to determine whether the variables had enough in common to warrant a PCA (Kaiser 1974). For each song type, we entered biologically relevant song traits into a discriminant function analysis (DFA) to determine if individuals could be distinguished from one another. Then, since the simple re-substitution table is an overly optimistic classification table, we tried the leave-one-out method, which shows a more realistic classification (StataCorp 2009). Assumptions of DFA, including multivariate normality and covariance, were verified. To investigate whether a male's type I song traits are similar to his type II song traits, we conducted a series of univariable linear regressions to compare the following:

type I song maximum frequency and type II song maximum frequency, type I song minimum frequency and type II song minimum frequency, type I song duration and type II song duration, and type I song FMA and type II song FMA.

We used a series of two-tailed paired *t*-tests to determine if, at a given nest, within-pair and extra-pair males differed in song variables, age, and body condition. When more than one extra-pair sire had paternity in a nest, we used the average of traits of all extra-pair sires to create an "average extra-pair sire" and to avoid pseudo-replication. To examine whether male fitness correlated with specific song traits, we conducted univariable linear regression to compare the total number of offspring sired with each song variable. Then, to examine whether male fitness correlated with song traits, we conducted a series of bivariable linear regressions again while controlling for age and then again while controlling for body condition.

All statistical analyses and procedures were done with Small Stata 11.0 (StataCorp LP 2009). The significance level of all statistical tests was $P < 0.05$. When we conducted multiple tests, we used sequential Bonferroni corrections (Rice 1989).

RESULTS

PATERNITY ANALYSES

In this study, we used a total of 17 nests with broods ranging from two to six nestlings (4.13 ± 1.25 nestlings per nest). We conducted paternity analyses on 67 nestlings and two embryos collected from unhatched eggs. Five nestlings and the embryos from the two unhatched eggs did not yield DNA and thus were not included in the analyses. We were able to identify 62 of these nestlings' sires (93%). At least one extra-pair young was found in 13 of 17 (76%) nests, and 24 of 62 (39%) nestlings had been sired by extra-pair males.

GENERAL SONG ANALYSIS

In the population we studied, males consistently sang type II songs with three syllable types but a varying numbers of notes per syllable (Highsmith 1989a, Fig. 1). While most males sang the type II song during the dawn chorus and the type I song during the day, one male sang the opposite pattern: type I song during the dawn chorus and type II song during the day. Because of this individual's "switched" song, we used his song in song-rate calculations only, not in any structural analysis. Also, seven males did not have a type I syllable A (the *zee* of the *zee-bee-bee-bee*). These seven males were excluded only from analyses involving this *zee* syllable (i.e., maximum and minimum frequency, duration, and FMA of type I syllable A) but were included in other analyses.

SONG VARIABILITY

Results from univariable Welch's ANOVAs comparing within- and among-male coefficients of variation in each song variable indicated significantly more variation among males than

TABLE 1. Descriptive statistics, coefficients, and results of Welch's ANOVA for each variable measured of the Golden-winged Warbler's song type I.

Variable	Mean \pm SD	Mean CV _w ^a (range)	CV _a ^b	<i>F</i>	<i>P</i>
Syllable A					
Maximum frequency	8.55 \pm 0.71	0.03 (0.01–0.09)	0.08	$F_{19,177} = 143.97$	<0.001
Minimum frequency	6.35 \pm 1.00	0.03 (0.01–0.11)	0.15	$F_{19,177} = 417.71$	<0.001
Frequency bandwidth	2.20 \pm 0.67	0.14 (0.07–0.23)	0.27	$F_{19,177} = 62.30$	<0.001
Duration	0.63 \pm 0.30	0.14 (0.04–0.27)	0.45	$F_{19,176} = 29.39$	<0.001
FMA	7.71 \pm 0.42	0.02 (0.01–0.05)	0.05	$F_{12,117} = 40.26$	<0.001
Syllable B					
Maximum frequency	7.64 \pm 0.39	0.03 (0.01–0.07)	0.04	$F_{19,175} = 28.09$	<0.001
Minimum frequency	5.34 \pm 0.35	0.03 (0.01–0.06)	0.06	$F_{19,175} = 65.87$	<0.001
Frequency bandwidth	2.29 \pm 0.50	0.12 (0.06–0.25)	0.18	$F_{19,175} = 26.91$	<0.001
Duration	0.34 \pm 0.08	0.15 (0.07–0.32)	0.20	$F_{19,175} = 64.28$	<0.001
FMA	6.39 \pm 0.52	0.07 (0.01–0.11)	0.05	$F_{18,171} = 4.75$	<0.001

^a Within-male coefficient of variation measuring the variation within a single recording of individual males.^b Among-male coefficient of variation calculated from the variable means of each male.

within individual males (Tables 1, 2; all $P < 0.001$). The PCA yielded four and five principal components (PC) with eigenvalues >1.0 , explaining 86% and 76% of the variation in the variables for song types I and II, respectively. However, the Kaiser–Meyer–Olkin measure of sampling adequacy indicated that the variables had an “unacceptable” amount in common to warrant a PCA (Kaiser 1974). Therefore, for each song type, we entered biologically relevant song traits into a DFA. For both song types I and II, a separate DFA using

maximum frequency, minimum frequency, and duration of each syllable was significantly better than chance at assigning the songs to the correct male (Table 3). For song type I, the first six canonical discriminant functions explained a significant amount of variation among the males, though the first three canonical functions (CAN1, CAN2, and CAN3) explained over 80% of the total variability (Table 3). For song type I, minimum frequency and duration of syllable A are the most important variables for CAN1, duration of syllable

TABLE 2. Descriptive statistics, coefficients, and results of Welch's ANOVA for each variable measured of the Golden-winged Warbler's song type II.

Variable	Mean \pm SD	Mean CV _w ^a (range)	CV _a ^b	<i>F</i>	<i>P</i>
Syllable A					
Maximum frequency	9.05 \pm 0.42	0.03 (0.02–0.05)	0.04	$F_{20,183} = 20.01$	<0.001
Minimum frequency	5.72 \pm 0.33	0.04 (0.01–0.07)	0.05	$F_{20,183} = 31.02$	<0.001
Frequency bandwidth	3.33 \pm 0.57	0.11 (0.06–0.25)	0.13	$F_{20,182} = 19.91$	<0.001
Duration	0.82 \pm 0.20	0.10 (0.03–0.25)	0.22	$F_{20,179} = 65.29$	<0.001
Trill rate	12.14 \pm 1.22	0.04 (0.02–0.08)	0.09	$F_{20,179} = 52.68$	<0.001
FMA	6.88 \pm 0.41	0.05 (0.01–0.11)	0.03	$F_{19,180} = 6.91$	<0.001
Syllable B					
Maximum frequency	8.80 \pm 0.42	0.03 (0.01–0.05)	0.04	$F_{20,182} = 21.37$	<0.001
Minimum frequency	5.61 \pm 0.26	0.02 (0.01–0.03)	0.04	$F_{20,182} = 62.91$	<0.001
Frequency bandwidth	3.19 \pm 0.41	0.09 (0.04–0.22)	0.09	$F_{20,182} = 12.77$	<0.001
Duration	0.40 \pm 0.60	0.12 (0.03–0.76)	1.20	$F_{21,193} = 182.17$	<0.001
Trill rate	8.09 \pm 1.41	0.11 (0.03–0.97)	0.15	$F_{21,193} = 10.65$	<0.001
FMA	6.47 \pm 0.88	0.10 (0.02–0.31)	0.08	$F_{20,189} = 16.55$	<0.001
Syllable C					
Maximum frequency	7.08 \pm 0.36	0.02 (0.01–0.05)	0.05	$F_{21,197} = 55.30$	<0.001
Minimum frequency	4.94 \pm 0.39	0.04 (0.02–0.09)	0.07	$F_{21,197} = 60.75$	<0.001
Frequency bandwidth	2.14 \pm 0.48	0.10 (0.04–0.18)	0.20	$F_{21,197} = 72.83$	<0.001
Duration	0.51 \pm 0.10	0.09 (0.04–0.15)	0.18	$F_{21,197} = 90.91$	<0.001
FMA	6.04 \pm 0.50	0.06 (0.01–0.10)	0.06	$F_{20,188} = 12.20$	<0.001

^a Within-male coefficient of variation measuring the variation within a single recording of individual males.^b Among-male coefficient of variation calculated from the variable means of each male.

TABLE 3. Results of the discriminant function analysis of type I and type II songs of the Golden-winged Warbler.

Canonical discriminant function	Eigenvalue	Approximate F^a	df		Proportion of variance	Cumulative proportion of variance
Type I						
1	0.97	33.53	114	980.3	0.52	0.52
2	0.95	23.82	90	829.2	0.26	0.79
3	0.88	16.63	68	673.4	0.09	0.88
4	0.85	13.35	48	512.4	0.08	0.96
5	0.73	8.61	30	346	0.03	0.99
6	0.55	5.36	14	174	0.01	1.00
Type II						
1	11.55	20.70	180.00	1396.00	0.39	0.39
2	6.37	16.73	152.00	1260.00	0.21	0.60
3	3.93	13.95	126.00	1120.00	0.13	0.73
4	3.05	11.88	102.00	976.20	0.10	0.83
5	2.24	9.77	80.00	827.50	0.08	0.91
6	1.05	7.64	60.00	673.60	0.04	0.94
7	0.72	6.79	42.00	514.00	0.02	0.97
8	0.63	6.23	26.00	348.00	0.02	0.99
9	0.31	4.58	12.00	175.00	0.01	1.00

^aIn all cases, $P < 0.0001$.

A and minimum frequency of syllable B are the most important variables for CAN2, and maximum frequency of syllable A and duration of syllable B are the most important variables for CAN3. Overall, however, it seems that duration of syllable A and maximum frequency of syllable B are the traits of type I song useful for distinguishing among males (Table 4). The DFA assigned 88% of the type I songs to the correct male, and when the leave-one-out method was used, 81% of the type I songs were assigned correctly.

For song type II, the first nine canonical discriminant functions explained a significant amount of variation among the males, though the first four canonical functions (CAN1, CAN2, CAN3, and CAN4) explained over 80% of the total variability (Table 3). For song type II, the minimum frequency of syllable B and maximum frequency of syllable C are the most important variables for CAN1, the maximum frequency and duration of syllable A are the most important variables for CAN2, the minimum frequency and duration of syllable C are the most important variables for CAN3, and the maximum frequencies of syllable B and C are the most important variables for CAN4. Overall, however, it appears that the maximum frequencies for each syllable are the traits of song type II useful for distinguishing among males (Table 4). The DFA assigned 93% of the type II songs to the correct male, and when the leave-one-out method was used, 84% of the type II songs were assigned correctly.

We compared differences between the maximum frequencies, minimum frequencies, duration, and FMA song types I and II song with univariable linear regressions. Only

the minimum frequencies of types I and II were significantly associated (linear regression, $F = 16.40$, $n = 177$, $P < 0.0001$) and remained significant after sequential Bonferroni corrections (critical $P = 0.0125$).

TABLE 4. Standardized canonical coefficients of the discriminant function for the Golden-winged Warbler's song types I and II.

Song type and trait	CAN1	CAN2	CAN3	CAN4
Type I				
Syllable A				
Maximum frequency	-0.34	0.02	0.75	
Minimum frequency	-0.75	-0.33	-0.26	
Duration	0.44	-0.96	0.03	
Syllable B				
Maximum frequency	0.15	0.34	0.33	
Minimum frequency	-0.18	-0.45	-0.26	
Duration	-0.15	-0.32	-0.50	
Type II				
Syllable A				
Maximum frequency	0.31	-1.28	-0.08	-0.03
Minimum frequency	-0.18	0.18	0.06	-0.36
Duration	0.09	-0.74	-0.15	0.07
Syllable B				
Maximum frequency	-0.07	1.30	0.39	-0.76
Minimum frequency	-0.71	-0.18	0.10	-0.29
Duration	-0.15	0.09	-0.36	-0.18
Syllable C				
Maximum frequency	-0.59	0.09	0.35	0.97
Minimum frequency	-0.01	-0.19	0.48	-0.29
Duration	-0.55	-0.12	-0.47	-0.29

RELATIONSHIPS BETWEEN SONG AND MATE CHOICE

A series of paired *t*-tests was conducted on 10 variables of song type I, 17 variables of type II, and on age and body condition. In none of these variables however, did the within- and extra-pair sires at a given nest differ significantly (paired *t*-test, all $P > 0.10$). To investigate females' mate choice further, we compared individual males' fitness (the total number of offspring sired) and song traits with linear regression. They differed significantly only in the minimum frequency of syllable B of song type I (linear regression, $F = 5.54$, $n = 16$, $P = 0.03$), and the difference was not significant after sequential Bonferroni corrections (critical $P > 0.00017$). A series of bivariable linear regressions on the number of offspring sired and song traits, controlling for age and body condition, revealed no significant associations ($P > 0.05$).

DISCUSSION

In both song types I and II, we found significantly more variability among male Golden-winged Warblers than within an individual male's repertoire. These findings suggest that the songs of individual male Golden-winged Warblers differ detectably. Other studies have found similar trends in another wood-warbler, the Chestnut-sided Warbler (*Dendroica pensylvanica*; Byers 1995, Lovell and Lein 2004, Wiley 2005, Lein 2008). The distinctiveness in song might be due to a copying error during song learning and/or might reflect genetic or territory quality (Nelson et al. 1996, Kroodsma et al. 2002). Consequently, vocal diversity can be enhanced by life histories or ecological variables (Nelson et al. 1996, Kroodsma et al. 2002) and can evolve via drift and selection (reviewed by Podos et al. 2004). Therefore, the distinctiveness of male Golden-winged Warblers' song may have arisen from a variety of factors and/or pressures.

Because male Golden-winged Warblers' songs vary distinctively, it is possible that females use the differences in song to differentiate among males or to evaluate male quality when choosing a mate. However, despite significant differences between characteristics of males' songs, we did not find evidence that females are using these differences when selecting mates, either within-pair or extra-pair.

Although our study suggests that female Golden-winged Warblers are not using the characteristics of the short type I and II songs we measured in their mate-choice decisions, it is important to note that Highsmith (1989a) found that the output of the long type I song drops sharply after a male pairs, suggesting that long type I and short type I may differ in function. Because the frequency of singing long type I decreases after males are paired, it is possible that long type I is used primarily for mate attraction and short type I serves a function more akin to song type II. Since our type I recordings were necessarily obtained after males had paired, we were limited to examining only the short type I song. If the function of short

type I is indeed primarily intrasexual, then it is not surprising that we found no significant relationship between song traits and mate choice.

Alternatively, a male may use the shortened song to communicate with his female partner (male Golden-winged Warblers often sing a quiet, short song type I when in close proximity to their paired female; R. Vallender, pers. obs.), even though continued advertisement to other females in the population (with whom they could engage in extra-pair copulations) would be expected. The role of the quiet type I song requires further examination and was beyond the scope of our study.

To date, several studies have found significant relationships between bird-song traits and paternity (Hasselquist et al. 1996, Leisler et al. 2000, Byers 2007), whereas other studies, including ours, have found no such relationship (Kroene et al. 1996, Marshall et al. 2007). Further research that directly compares the songs of multiple males chosen by the same female during the same breeding attempt (i.e., comparing within- and extra-pair males) is needed in order to gain a better understanding of the role that song plays in females' choice of mates.

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