



Evidence of honest signalling in a suboscine bird: Southwestern willow flycatcher song frequency is positively related to apparent reproductive success

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Abstract

Acoustic signalling mediates key animal behaviours, but the factors driving inter- and intra-specific variation remain elusive, especially in non-learning suboscine birds. Acoustic traits may evolve to signal an individual's quality through a process termed honest signalling. Most studies on bird song evolution via honest signalling focus on oscine bird species (song learners) while fewer evaluate honest signalling in suboscines. We tested whether song was an honest signal of male quality in southwestern willow flycatchers (*Empidonax traillii extimus*), a suboscine, by monitoring nest success and recording male advertisement songs. Based on song characteristics known to vary among individuals, we found higher minimum song frequency, but not temporal components, was

positively correlated with the number of fledglings and nest success, after accounting for first egg date, the number of eggs, and clutch number. Our study therefore offers evidence that suboscine song may have evolved as an honest signal of reproductive success.

Keywords

honest signal, reproductive success, sexual selection, song, suboscine, Tyrannidae.

1. Introduction

Song is used by birds to attract mates (Collins, 2004), but the reasons why particular songs are selected in mating decisions, and the evolutionary mechanisms mediating preferred song traits, remain unresolved (Andersson, 1994). Song characteristics may evolve through genetic drift, natural selection, sexual selection, cultural selection, arbitrary mate choice, or a combination of these factors (Prum, 2010). Under the sexual selection model, mating decisions are based on preferred traits, and numerous studies suggest song is an honest signal of reproductive success, that is, song indicates quality to potential mates (reviewed in Soma & Garamszegi, 2011, but see Garamszegi & Møller, 2004; Byers & Kroodsma, 2009). Mates may choose songs that signal higher quality (Baker et al., 1986), as this increases the mate's own fitness directly and/or indirectly (Andersson, 1994; Johnstone, 1995; Kokko et al., 2003). If songs signal quality, song traits should then be correlated with an individual's quality such as reproductive output (McGregor et al., 1981; Kokko et al., 2002; Safran et al., 2013).

A meta-analysis assessing the relationship between song and genetic and social (apparent) reproductive success found song predicted the number of mates and offspring; however, this was biased towards oscine songbirds (Soma & Garamszegi, 2011). Studies of suboscines found nest initiation, participation during dawn chorus (Sexton et al., 2007; Murphy et al., 2008; Bruni & Foote, 2014), and song structure (Garamszegi, 2005; Araya-Ajoy et al., 2009) predicted mate quality, reproductive and/or mating success, and brood numbers. Generally, however, whether suboscine song characteristics signal reproductive success is less understood compared to song learners (but see Araya-Ajoy et al., 2009). Song characteristics such as frequency and temporal aspects may be selected to optimize transmission given the ecological context (Marten & Marler, 1977; Marten et al., 1977) or because they signal mate quality, or both, or they may be arbitrarily selected (Prum, 2010). Suboscines are a useful group in which to explore the role of sexual selection in shaping song characteristics because song variation is not confounded

by other sources of variation such as cultural learning (Tobias et al., 2012). Therefore, if song is related to reproductive success, this may be strong evidence that song is an honest signal of mate quality (Safran et al., 2013).

The southwestern willow flycatcher (*Empidonax traillii extimus*) is a subspecies of the willow flycatcher that occurs in the Southwestern United States. Willow flycatchers are suboscines with no evidence of song learning (Kroodsma, 1984). Their main advertising vocalization is the ‘fitz-bew’ song (Figure 1), which varies among and is unique to individuals (Fernández-Juricic et al., 2009). In studies assessing geographic song variation in willow flycatchers, minimum song frequencies and frequency modulations of the song’s final notes explained the most variation among individuals (Figure 1; Sedgwick, 2001; Mahoney et al., 2020), but the factors mediating individual variation remain unknown. Larger bodied tyrannid species with heftier bills sing lower frequency and slower paced songs (Schoen et al., 2023), however body size (estimated by wing chord) and bill morphology (length and width) do not vary widely among willow flycatcher individuals (male museum specimens vary on average 4% for wing chord, 3% for bill width, and 5% for nares length; Unitt, 1987), suggesting an alternative factor is shaping song in this species. Sex ratios in willow flycatchers range from 40% female and 60% male to 71% female and 29% male (Paxton et al.,

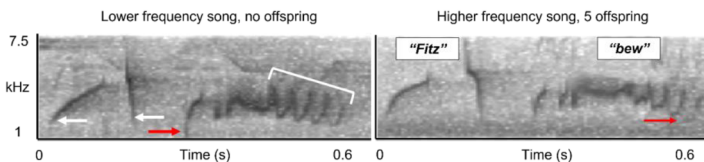


Figure 1. Southwestern willow flycatcher (*Empidonax traillii extimus*) ‘fitz-bew’ songs are their main advertising vocalization. Individuals that sang lower frequency songs (left panel) produced fewer offspring than individuals that sang higher frequency songs (right panel, $N = 20$). Five labile song elements were measured following Mahoney et al. (2020) and are indicated by arrows and a bracket. From left: Phrase 1 note 1 minimum frequency (Hz), phrase 1 note 2 minimum frequency (Hz), phrase 2 note 1 minimum frequency (Hz, arrows), and frequency modulation count (bracket). Red arrows indicate the fifth song element, overall minimum frequency in the song. Phrase 2 note 1 minimum frequency on left panel is also the lowest frequency in song, therefore the red arrow denotes both characteristics. Recordings are from different areas, but study site explained relatively little variation in our analyses ($\sigma^2 = 0.06$). The lower frequency exemplar was recorded at the Gila River near Safford, AZ, USA by author SMM, and the higher frequency exemplar was from the Seegmiller Marsh near St. George, UT, USA recorded by author DR. Recordings included as supplemental files.

2002), indicating sexual selection may be acting within some populations. Predicting reproductive success acoustically would be important from an applied perspective as well, because practitioners could remotely identify fecund pairs. We tested the honest signalling hypothesis in southwestern willow flycatchers and specifically assessed if minimum song frequencies and frequency modulations (shown to vary among willow flycatcher individuals, Mahoney et al., 2020) were correlated with reproductive success.

2. Methods

2.1. Nest monitoring

We monitored southwestern willow flycatcher nests ($N = 20$) throughout the breeding season (May–July) in 2016 through 2018 at 4 locations spanning the subspecies' range. In 2016, we monitored nests along the Gila River near Pima, AZ, USA ($N = 6$) and at Seegmiller Marsh near St. George, UT, USA ($N = 5$), and at Alamo Lake, AZ, USA ($N = 5$) in 2017. In 2018, we monitored nests at Elephant Butte Reservoir, NM ($N = 4$). Territories were identified by playback survey (Sogge et al., 1997). We used a standardized methodology specific to southwestern willow flycatchers to locate nests following Rourke et al. (1999) by searching the area and observing adults returning to the nest (English et al., 2006). We found 11 nests while the female was building, one nest during egg laying, six nests during incubation, one nest during nestling phase, and one individual was unpaired. All birds from Seegmiller and two from Alamo Lake were colour-banded, thus those individuals could be resighted at territories and there were no instances of territory replacement at those territories. Birds from Elephant Butte, the Gila River, and three birds from Alamo Lake were not banded. While it is possible the territory holder was replaced during the breeding season, territory fidelity is high (Sedgwick, 2004; Koronkiewicz et al., 2006), switching territories is costly (Sedgwick, 2004), and most mortality typically occurs during migration, based on long term monitoring (Paxton et al., 2007). Therefore, at Elephant Butte, the Gila River, and at a subset of Alamo Lake territories, we assumed the individuals initially identified as territory holders held the territory throughout the breeding season and deviations from this assumption should diminish statistical effects. While it is possible that we did not detect every nest associated with a particular male in the study site, we do not think this is likely given our rigorous nest checking methods. Field crews consisted

of 2–20 technicians per year, and after the nest was located, we checked it every 2–4 days. During nest checks, we used a mirror fixed to a telescoping pole and counted the contents of the nest (eggs and/or nestlings) and counted fledglings. We determined the fate of each nest based on whether the nest fledged at least one young (i.e. successful nest) or if the nest failed to fledge at least one young (i.e. failed). This determination was based on any of four criteria used in southwestern willow flycatcher nest monitoring (English et al., 2006; Paxton et al., 2007): (1) observers viewed fledglings leaving the nest or were observed near the nest; (2) adults were observed feeding flycatcher fledglings; (3) nests were empty within 2 days of estimated fledging date; (4) if nests were empty, adults exhibited defensive behaviour after the estimated fledging date. In one case, a pair from Alamo Lake had two successful broods. Two males from Seegmiller were polygynous and produced respectively five and two successful broods. Two males from Alamo Lake were also polygynous and produced one and four fledglings respectively. Six pairs produced a second clutch after the first nesting attempt failed. One male was unpaired and therefore produced 0 eggs, nestlings, and fledglings. The remaining nine pairs produced one clutch (Data available upon acceptance). To estimate reproductive success, we summed the number of eggs, nestlings, and fledglings from each nest for a single value respectively for that individual. There were two nests that were parasitized by brown-headed cowbirds (*Molothrus ater*) and subsequently, the parasitized nests were treated as unsuccessful nests. As in similar studies assessing the relationship between secondary sex characteristics and reproductive success, we did not determine paternity of offspring, so our estimates are based on social (apparent) reproductive success (Reid et al., 2005; Brunton et al., 2016). Willow flycatcher extra-pair paternity rates are estimated to be 14% (range = 0.03–0.32, Pearson et al., 2006). While behaviourally polygynous males sire more young than behaviourally monogamous males, this is not offset by differences in extra-pair paternity (Pearson et al., 2006). Therefore, while some level of extra-pair reproduction occurs and could alter the results, behavioural estimates of reproductive success adequately capture reproductive success in southwestern willow flycatchers (Pearson et al., 2006).

2.2. Song recordings and analyses

We recorded singing male southwestern willow flycatchers (from 10 m distance) in each focal territory with a Sennheiser ME66 shotgun microphone (mono-line) with Rycote handgrip and Rycote Softie windshield and

a Marantz PMD661 MKII solid-state recorder (44.1 kHz sampling rate). To avoid recording the same individual twice, we only recorded individuals separated by at least 200 m. We identified an individual's sex based on behaviour; while females are reported to sing (Seutin, 1987; Yard & Brown, 2003), males can be behaviourally identified because they advertise from conspicuous territorial perches and females sing less often and typically in response to territory intrusions close to their nest (M.A. McLeod, pers. commun.). Therefore, we restricted our recordings to songs that were unsolicited (i.e., playback was not used to elicit a song) and from individuals displaying on conspicuous perches (Mahoney et al., 2020, 2021). We then quantified frequency (Hz) and temporal song elements identified by Mahoney et al. (2020) to be variable among individuals. In that study, the authors found the following five song elements explained the most variation from a principal components analysis (PCA): minimum overall frequency; low frequencies in phrase 1 note 1, phrase 1 note 2, and phrase 2 note 1; and the minimum number of frequency modulations in the terminal portion of the song. Therefore, to test the relationship between male quality and song, we quantified the frequency and temporal song components as above (Figure 1). We digitized song recordings using Raven Pro (Cornell Lab of Ornithology) using the Hann Window (spectrogram visualization parameters: size = 256 samples, 50% window overlap, DFT = 256 samples; Mahoney et al., 2020) with an unsmoothed view. To minimize background noise, we used a high- (at 1 kHz) and low-pass (at 7.5 kHz) filter, and we only used recordings with high signal-to-noise ratios. We selected the first high signal-to-noise song in the recording, and we used one song from each individual (as in Sedgwick, 2001; Mahoney et al., 2020; Schoen et al., 2023), because intra-individual song variation in southwestern willow flycatchers is low and repeatable (Fernández-Juricic et al., 2009). To confirm songs in our study were repeatable, we quantified three songs per individual as above, and conducted a repeatability analysis using the R (R Core Development Team, 2023) package *rptR* (Stoffel et al., 2017). One individual from Alamo Lake had two recordings. Another individual from Seegmiller had one high signal-to-noise song recording, thus that individual was removed from the *rptR* analysis. We found all song variables were repeatable (Table A1 in the Appendix).

While morphology can influence song frequency and temporal elements in some Tyrannidae species (Schoen et al., 2023), our song characteristics are not corrected for body size. Willow flycatcher morphology, specifically

body size and bill morphology, do not vary widely among individuals (Unitt, 1987), and we assumed morphology does not influence song characteristics. To confirm this assumption, we tested the relationship between song and morphology using an unpublished dataset of willow flycatcher song and morphology. As part of an ongoing project, we recorded songs from banded individuals near Moab, UT, USA ($N = 8$ individuals). Immediately following the song recording, we captured the individual. There were no instances when more than one individual was in the vicinity of the net when an individual was recorded and captured; thus, we are confident the song came from a known individual. We then measured body mass (g) using hanging scales, wing chord (mm) with a wing ruler, and tarsus length (mm), bill length (from nares), and bill width (mm) using callipers. We quantified five song aspects from recordings as above and confirmed song characteristics were repeatable from a repeatability analysis in the R package *rptR* ($R = 0.51$, $p = 0.047$; $\bar{x} = 2.4$ songs/individual). We then summarized the song and morphology characteristics using a PCA and we used linear regressions to test the relationship between song PC1 and body size PC1 (Table A2 in the Appendix). We multiplied PC scores by -1 to help with interpretation. We found no relationship between song PC1 and body size PC1 ($R^2 = 0.12$, $p = 0.21$), however we emphasize this is based on few individuals from a different range. Future work should more rigorously assess the relationship between body size, bill morphology, and song variation to help unravel the factors involved in willow flycatcher song evolution.

2.3. Data analysis

To assess the relationship between song and apparent reproductive success in southwestern willow flycatchers, we summarized the song frequency and temporal elements from above using a PCA and selected principal components (PCs) with eigenvalues > 1 . We then constructed linear mixed effects models using the *lme4* package in R (Bates et al., 2015) testing the relationship between the total number of fledglings as the response variable, song characteristics (summarized with PCA) and the first egg laying date (Julian day of year) as main effects. In six cases, the date of the first laid egg was unknown and we estimated the egg laying date by back calculating from the last observed phase of the nest based on the duration of each nesting phase (two days for egg laying; 12 days for incubation; 13 days for nestling; a total of 27 days to fledge) estimated from 8 years of extensive nest monitoring

on the Lower Colorado River, including at our Alamo Lake site (McLeod & Pelligrini, 2011). Of the six cases with unknown first egg dates, four nests fledged young, and we estimated the first egg date by subtracting 27 days from the fledge date. In one instance, the last observed phase was incubating, so we subtracted 14 days. For the final nest, the last observed nest phase was egg laying and we subtracted two days to estimate first egg date. Similar methods have been used in our study areas to calculate first egg laying dates (Dobbs et al., 2012).

There may be a positive relationship between the number of nesting attempts and reproductive success in willow flycatchers as pairs will re-nest following failed attempts (Sedgwick, 2004). Indeed, we found positive relationships between the number fledglings and eggs, nestlings, and clutches (eggs: $\chi^2 = 31.1$, $p < 0.0001$, site $\sigma^2 = 0.08$; nestlings: $\chi^2 = 77.3$, $p < 0.0001$, site $\sigma^2 = 0.0$; clutches: $\chi^2 = 4.75$, $p = 0.03$, site $\sigma^2 = 0.34$) and the number of eggs and nestlings were highly correlated ($R^2 = 0.80$, $p < 0.0001$). Therefore, we included the total number of eggs and clutch number as main effects in the model, consistent with similar studies of reproductive success (Woodgate et al., 2012). Site was included as a random effect in all models. We conducted the analysis again excluding the polygynous males (see Section 2.1) to ensure the results were not inflated by their relatively high reproductive success.

In separate models, we tested if song characteristics (from PCA) predicted overall reproductive success (i.e. nests that fledged or failed) using a logistic regression model. Nest success was included as the response variable (coded as 0 for a nest that did not fledge young and 1 for a nest that fledged $n \geq 1$ young, see *Nest Monitoring* methods), and main and random effects were included as above. The number of eggs, nestlings, and fledglings did not vary annually (ANOVA $F_{1,18} = 3.12$, $p = 0.09$; $F_{1,18} = 1.26$, $p = 0.28$; $F_{1,18} = 0.08$, $p = 0.79$, respectively), thus we did not include year in our models. The nesting phase during which we located nests was not related to nest success (fledglings: $\chi^2 = 0.9$, $p = 0.82$; overall success: $\chi^2 = 1.36$, $p = 0.72$); subsequently phase was not included in the models. Due to multiple hypothesis tests, we calculated adjusted p -values using a 5% false discovery rate. We scaled values prior to all analyses by subtracting the mean value and divided by the standard deviation, to meet assumptions of normality and homoscedasticity, and models were fit using a Gaussian error distribution. We calculated p -values using likelihood ratio tests using the *car* package in

R (Fox et al., 2012). Finally, we calculated effect sizes (η^2) for all model results using the *effectsize* package in R (Ben-Shachar et al., 2020).

2.4. Ethical notes

We conducted all field work in accordance with federal and state laws and in compliance with institutional animal care and use guidelines.

3. Results

From our song PCA, PC1 explained 49% of the variation in song structure (eigenvalue = 2.46), and song PC2 explained 22% of the total variation (eigenvalue = 1.10). PC1 and PC2 had eigenvalues >1, so we used them in subsequent analyses (Table 1). Song PC1 was positively correlated with minimum song frequency, and to a lesser extent, positively related to minimum frequency in phrase 1 note 2 and phrase 2 note 1 (Table 1). PC2 was most strongly and positively correlated with frequency modulations, but also to minimum frequencies in phrase 1 note 1 (Table 1). Therefore, higher PC1 and PC2 scores represented songs with higher minimum frequencies and with more frequency modulations in the terminal portion of the song.

Table 1.

Principal components analysis results summarizing southwestern willow flycatcher (*Empidonax traillii extimus*) song variation.

Variable	PC1	PC2	PC3	PC4	PC5
Variation					
Standard deviation	1.57	1.05	0.90	0.70	0.37
Eigenvalue	2.46	1.10	0.81	0.49	0.14
Proportion of variance	0.49	0.22	0.16	0.10	0.03
Cumulative proportion	0.49	0.71	0.88	0.97	1.00
Component loadings					
Min. song frequency	<i>0.57</i>	-0.32	0.02	-0.15	0.74
Phrase 1 note 2 min. frequency	<i>0.51</i>	0.11	0.03	0.83	-0.17
Phrase 2 note 1 min. frequency	<i>0.55</i>	-0.30	0.03	-0.43	-0.64
Frequency modulations	0.21	<i>0.68</i>	0.66	-0.23	0.06
Phrase 1 note 1 min. frequency	0.25	<i>0.58</i>	-0.75	-0.20	0.04

PC1 was associated with minimum song frequency, phrase 1 note 2 minimum frequency, and phrase 2 note 1 minimum frequency (Hz). PC2 was associated with temporal components (count of frequency modulations), and phrase 1 note 1 minimum frequency, shown in italics.

The total number of fledglings produced was positively related to song PC1 (Figure 2A; $\chi^2 = 4.66$, $p = 0.03$, site $\sigma^2 = 0.06$), after accounting for first egg date ($\chi^2 = 0.45$, $p = 0.50$) and the number of clutches ($\chi^2 = 0.03$, $p = 0.87$; Table 2). Fledglings were also positively related to the number of eggs (Figure 2B; $\chi^2 = 5.50$, $p = 0.02$). The effect of song PC1 was consistent when the polygynous individuals were removed from the analysis (fledglings and song PC1: $\chi^2 = 5.88$, $p = 0.005$, site $\sigma^2 = 0.0$; all other predictors $p > 0.10$), however the number of eggs no longer predicted the number of fledglings ($\chi^2 = 1.45$, $p = 0.23$), indicating the effect of song PC1 was not biased by the relatively high number of offspring produced by polygynous males. The number of fledglings was not related to song PC2 ($\chi^2 = 0.003$, $p = 0.95$, site $\sigma^2 = 0.23$; Table 2).

Overall reproductive success was positively related to song PC1 (Figure 2C; $\chi^2 = 8.00$, $p = 0.005$, site $\sigma^2 = 0.55$), after accounting for first egg date ($\chi^2 = 0.16$, $p = 0.69$), the number of eggs in the nest (Figure 2D; $\chi^2 = 0.15$, $p = 0.70$), and the number of clutches ($\chi^2 = 0.0003$, $p = 0.97$; Table 2). Our results were not offset if we removed polygynous individuals from the analysis (overall success and song PC1: $\chi^2 = 18.30$, $p < 0.0001$, site $\sigma^2 = 0.4$; all other predictors $p > 0.28$), indicating polygyny did not bias the relationship. Overall reproductive success was not related to song PC2 ($\chi^2 = 0.04$, $p = 0.84$, site $\sigma^2 = 0.36$; Table 2).

4. Discussion

Song is used to attract mates, and therefore is often thought to have evolved to signal quality via honest signalling (Soma & Garamszegi, 2011). Under the honest signal model of song evolution, individuals of higher quality exhibit song characteristics that are correlated with measures of quality, such as the number of young produced (Safran et al., 2013). Studies evaluating song and quality often focus on oscine songbirds, where song is learned and therefore thought to be relatively more plastic than that of suboscines, where song is innate (Raposo & Höfling, 2003). In our study, we found minimum song frequencies, a labile song characteristic among suboscine southwestern willow flycatcher individuals (Mahoney et al., 2020), predicted the apparent reproductive success of males after controlling for the first egg date, the number of eggs laid, and the number of clutches.

A logical alternative hypothesis to explain reproductive success is that pairs which lay more eggs will produce more fledglings, but there may

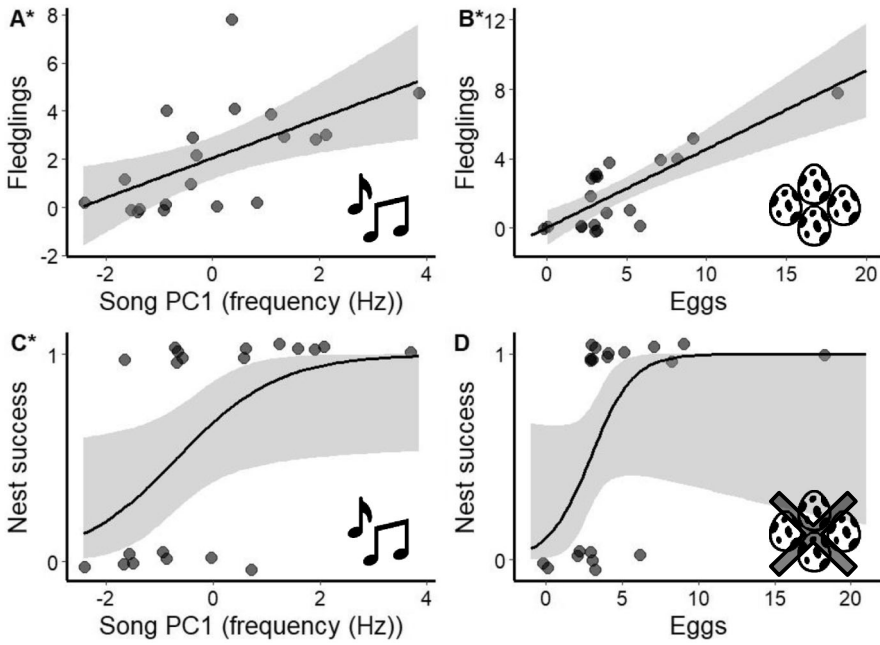


Figure 2. Southwestern willow flycatchers (*Empidonax traillii extimus*) songs predicted the number of offspring. Individuals that (A) sang higher minimum song frequencies (Hz, summarized by PC1, 49%, eigenvalue = 2.46) and (B) had mates that laid more eggs, produced more fledglings. (C) Individuals that sang higher frequency songs also had more successful nests (fledged ≥ 1 offspring); however, (D) eggs did not predict whether nests were successful. Schematics refer to dependent variables. Asterisks indicate significant effects after false discovery rate adjustments. Larger song PC1 values represent songs with higher minimum frequencies. Raw data are plotted and points are jittered to help with presentation.

be a trade-off between egg quantity and quality (Smith & Fretwell, 1974). Under experimentally increased clutch sizes, nests with more eggs experienced lower nest success, thus laying more eggs does not necessarily increase reproductive success (Nager et al., 2000). Indeed, we found mixed evidence that eggs correlated with reproductive success. While the number of eggs laid predicted the number of fledged offspring, when assessing if the nest was successful or not (whether a nest fledged ≥ 1 young), eggs no longer predicted success, but song PC1 did. Additionally, the effect of eggs was dependent on the relatively high number of eggs from polygynous individuals, whereas the song PC1 effect was consistent regardless of whether polygynous individuals were included in the analysis.

Table 2.

Southwestern willow flycatcher (*Empidonax traillii extimus*) males that sang advertisement songs with higher minimum frequencies (song PC1) produced more fledglings and had higher reproductive success, after accounting for nest initiation date, number of eggs laid, and number of clutches.

Response	Predictor	χ^2	df	<i>p</i>	η^2	σ^2
Fledglings	PC1	4.66	1,19	0.03	0.38 [−0.01, 0.77]	0.06
	First egg date ¹	0.44	1,19	0.50	−0.10 [−0.42, 0.23]	
	Eggs	5.50	1,19	0.02	0.64 [0.04, 1.23]	
	Clutches	0.03	1,19	0.87	−0.04 [−0.61, 0.53]	
Success ²	PC1	8.00	1,19	0.005	0.62 [0.14, 1.11]	0.55
	First egg date	0.16	1,19	0.69	−0.07 [−0.47, 0.32]	
	Eggs	0.15	1,19	0.70	0.13 [0.60, 0.85]	
	Clutches	0.0003	1,19	0.99	0.005 [−0.69, 0.70]	

Song PC2 (temporal elements) was not related to reproductive success. False discovery rate adjusted significant effects from linear mixed effects models are shown in italics. Effect sizes (η^2 , 95% CIs) for each term and random effect variance of site (σ^2) are included.

¹ First egg date refers to Julian date (day of year from 1 January).

² Success refers to overall reproductive success (coded as 0 for no fledglings or 1 for ≥ 1 fledgling in logistic regression model).

Other aspects of suboscine song vary with individual quality including participation in dawn chorus (Sexton et al., 2007; Murphy et al., 2008) and duetting (Diniz et al., 2019), however far fewer studies found a relationship between song elements (such as frequency characteristics) and reproductive output (Araya-Ajoy et al., 2009). An important caveat of our study is that our estimates of reproductive success do not account for extra-pair paternity; therefore, reproductive success may be overestimated for some individuals and underestimated for others. Southwestern willow flycatcher extra-pair paternity rates were estimated to be 14% (range = 0.03–0.32) in a population on the Kern River, CA, USA (Pearson et al., 2006). However, polygynous males sired more young than behaviourally monogamous males, and this was not offset by differences in extra-pair paternity (Pearson et al., 2006), suggesting behavioural estimates of reproductive success, as we used, adequately capture reproductive success.

Southwestern willow flycatchers are riparian obligates, often nesting in willows (*Salix* spp.) and tamarisk (*Tamarix* spp.), and the vegetation in their environment may impact the transmission of their songs (Morton, 1975; Seddon, 2005). Individuals that produced one or more fledglings exhibited

minimum song frequencies above 1526 Hz on average, and we hypothesize this song frequency transmits optimally for receiver detection within riparian environments; however, attenuation playback experiments are needed to confirm this. Alternatively, individuals with successful nests may have been singing louder to increase receiver detection, which can increase song frequency (Nemeth et al., 2013). The highest frequency song in our dataset was from the Seegmiller Marsh in St. George, UT, USA and the individual was adjacent to a road, while the lowest frequency song was from a more rural site at the Gila River near Safford, AZ, USA. It is possible that anthropogenic noise may have impacted our results. If birds were responding to variation in anthropogenic noise, it follows that average song frequencies would be highest at the more urban Seegmiller site and lowest at the rural Gila River and Alamo Lake sites. In contrast to this notion song PC1 did not vary among sites (ANOVA $F_{3,16} = 2.91$, $p = 0.07$), and site explained little variation in our models of reproductive success (fledglings: $\sigma^2 = 0.06$; overall nesting success: $\sigma^2 = 0.55$). Therefore, we do not think anthropogenic noise in our sites influenced our results. Future work is needed to determine if tyrant flycatchers increase minimum song frequencies to avoid signal overlap with vehicle traffic (Gentry et al., 2018).

In other species, song communicates territory quality to potential mates (Buchanan & Catchpole, 1997; Scales et al., 2013; Manica et al., 2014). The reasons behind this relationship include higher quality territories may hold better resources to support nesting females, the vegetation may be such that nests are more likely to be concealed from predators, and/or territory holders may be more aggressive and territorial (Phillips et al., 2020). Thus, there is selection pressure to sing unique songs so competitors can avoid costly agonistic interactions and females can easily identify individuals holding high quality territories (Phillips et al., 2020). Further, signalling territory quality may be one mechanism to prevent cheating. While signalling theory predicts that lower quality individuals should deceive potential mates and competitors by cheating (Maynard Smith, 1982), deception can result in costly territorial fights (Molles & Vehrencamp, 2001). Therefore, signals evolve to maintain honesty (Maynard Smith & Harper, 1988). Although we did not monitor arrival dates or initial pairing dates, individuals from our study singing higher frequencies songs may have held higher quality territories. The primary objective of our study was to assess the connection between

flycatcher song and a direct male quality measure (i.e. number of young produced) and future studies should assess the specific information encoded in southwestern willow flycatcher song that is signalling reproductive success.

There is some evidence that singing is a physiologically demanding behaviour, and there may be a tradeoff between the energy required to sing given an animal's morphology (Podos et al., 2004). Body mass and bill morphology can influence song characteristics in birds generally (Mikula et al., 2021), and tyrant flycatchers specifically (Schoen et al., 2023). Based on willow flycatcher individuals from Moab, UT, morphology was not related to song characteristics, however we did not have morphology for all individuals in our study with reproductive success estimates. Although it remains untested in southwestern willow flycatcher individuals, if bill and body size constrain vocal characteristics, our results suggest singing at higher minimum frequencies represents a performance challenge that is under sexual selection (Zahavi, 1975; Podos, 1997; Luther et al., 2016). Further, the interaction between bill morphology and song structure could signal foraging capabilities such as in Galapagos finches (Christensen et al., 2006). Alternatively, the higher frequency songs may be preferred by females through arbitrary mate choice (Prum, 2010). Future work should assess the role of morphology in song variation in willow flycatchers to disentangle the drivers of song evolution in this species.

Older birds may be more successful breeders, and age may be encoded in song signals (Kipper & Kiefer, 2010). A comprehensive study of song sparrows (*Melospiza melodia*) found repertoire size was positively correlated with the number of offspring and grand offspring; however, older individuals exhibited songs with larger repertoires (Reid et al., 2005). Similarly, older individuals can sing lower frequency songs (Garamszegi et al., 2005). Future studies could assess this hypothesis as we were not able to determine ages of individuals, but it is possible that song signals age, which can be correlated with reproductive behaviour (Kipper & Kiefer, 2010). Long term monitoring of southwestern willow flycatcher populations and their vocal behaviour will be needed to assess the relationship between age, song, and reproductive success.

Acoustic monitoring is a useful tool in conservation as it may allow researchers to remotely study key behaviours mediating the life histories of endangered animals (Teixeira et al., 2019). An on-going management challenge in the U.S. is the conservation of southwestern willow flycatcher

populations (Ruegg et al., 2018). Threats to the existence of willow flycatchers in the southwest are numerous and complex but are mostly attributed to riparian habitat loss (FR, 1995; 60 FR 10694-10715). Given the results of our study, we recommend acoustic monitoring of southwestern willow flycatcher populations accompany traditional nest monitoring surveys to evaluate the reproductive success of breeding pairs.

Song is an important behaviour in birds, but the evolutionary factors involved in suboscine song variation are unresolved (Tobias et al., 2012). While it is thought that suboscine song plasticity is relatively low, there is some evidence that individual singing behaviour is an honest signal (e.g. dawn chorus participation (Murphy et al., 2008) and duetting (Diniz et al., 2019)). We offer evidence that suboscine song structure is an honest signal of reproductive success, because minimum song frequencies, which are variable among individuals, predicted the number of fledglings. While the specific mechanism remains to be studied, our study offers unique evidence of the role of honest signalling in suboscine song evolution.

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Appendix

Table A1.

Southwestern willow flycatcher songs are significantly repeatable, based on repeatability analyses.

Variable	<i>R</i>	Lower CI	Upper CI	<i>p</i>
Phrase 1 note 1 minimum frequency	0.66	0.39	0.83	<0.0001
Minimum song frequency	0.49	0.16	0.68	0.004
Phrase 1 note 2 minimum frequency	0.44	0.13	0.68	0.001
Phrase 2 note 1 minimum frequency	0.52	0.23	0.74	0.0001
Frequency modulations	0.86	0.72	0.93	<0.0001

Higher *R* values indicate song variable is more repeatable within individuals. We used three songs per individual in analyses; however, one individual had $N = 2$ recordings. One individual was removed from analyses because only one song was recorded.

Table A2.

Principal components analysis loadings of willow flycatcher (*Empidonax traillii*) song from banded individuals with morphology measurements.

Variable	PC1	PC2	PC3	PC4	PC5
Song					
Phrase 1 note 1 minimum frequency	0.27	−0.43	<i>0.82</i>	0.28	−0.01
Minimum song frequency	0.03	0.31	0.44	−0.85	0.01
Phrase 1 note 2 minimum frequency	<i>0.79</i>	−0.40	−0.36	−0.30	0.00
Phrase 2 note 1 minimum frequency	<i>0.56</i>	<i>0.75</i>	0.10	0.35	0.00
Frequency modulations	0.00	0.00	0.00	0.01	1.00
Variation	0.84	0.1	0.05	0.01	0
Eigenvalue	20.99	12.39	10.32	7.34	0.88
Morphology					
Mass	0.34	−0.91	0.12	0.19	0.01
Bill length (nares)	0.07	0.23	0.16	<i>0.86</i>	−0.43
Bill width	−0.09	0.06	−0.07	0.45	<i>0.89</i>
Wing chord	0.15	−0.05	−0.97	0.12	−0.12
Tarsus length	−0.92	−0.33	−0.09	0.11	−0.13
Variation	0.59	0.3	0.09	0.01	0.005
Eigenvalue	1.70	1.45	1.05	0.62	0.54

Data collected from Moab, UT, USA in May 2023. False discovery rate adjusted significant effects from linear mixed effects models are shown in italics. All animals were handled in accordance with federal and state laws and in compliance with institutional animal care and use guidelines.