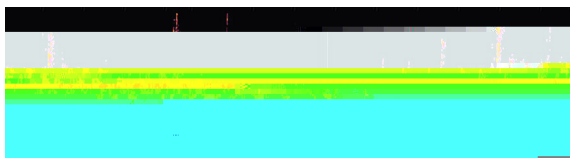
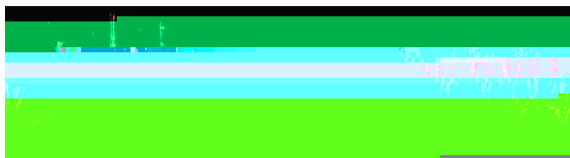
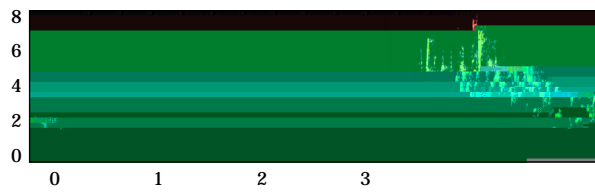
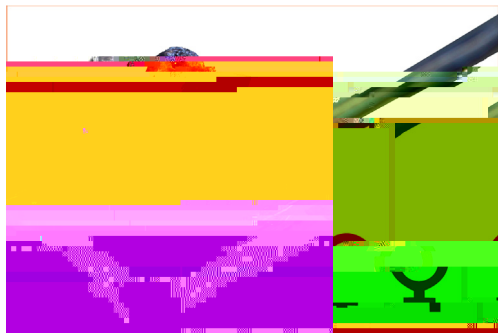


Analysis of female song provides insight into the evolution of sex

([Brown & Brown, 2020](#)), but no formal descriptions or quantitative analyses of that song exist (see [Supplementary Methods and Background](#) for clarifying notes on female song).

General Field Methods



E

Characterizing Female Song

Recordings and observations revealed that females used a distinctive vocalization in spontaneous solo renditions, and in response to hearing other females, in a manner synonymous to male countersinging. Females were also observed using the vocalization to interrupt the songs of their mates (see video at <https://vimeo.com/424642268>). This vocalization is relatively complex, including many syllables with similar frequency modulation and pace to male song (Fig. 1). Collectively, this evidence confirms that female barn swallows do produce facultative song, rather than just simple calls (Langmore, 1998).

Female song bouts were often produced within the nest, but also on perches near the nest or outside the barn/structure, and were usually short and infrequent. As such, only 78 songs were recorded from 18 identifiable females over the course of 2 years, compared to 753 (865% more) songs from 40 males, given the same recording effort (~105 h). This amounts to a recording efficiency of about 7.17 clear, identifiable songs/h for males and 0.74 songs/h for females over the course of the study. The sexes also differed strikingly in the phenology of song production. Male songs were observed and recorded between 1 May and 21 August, while female songs were only observed and recorded between 10 May and 29 May (pooling years) (Fig. 2a). Restricting to only the active singing period for females during May of both years, a cumulative 91 h of recording documented 8.9 individually identifiable songs/h for males and 0.86 songs/h for females. We could not calculate exact song rates per individual due to the complexities of recordings with shifting focal singers in dynamic colonial environments. To explore effects of breeding phenology on singing output, we also calculated

relative recording dates by subtracting the ordinal date of first clutch initiation (i.e. breeding onset) from the ordinal recording date. For males, these relative recording dates ranged from 59 days before to 109 days after the first egg was laid by a male's mate, with

Trait means, sample sizes and sex difference confidence intervals are reported in the Appendix, [Table A2](#).

Connecting Contemporary Selection to Phenotypic Sex Differences

Our final objective was to test whether sexual differentiation is more strongly associated with contemporary selection in males or females (here, using surrogate measures of lifetime fitness: within-pair reproductive performance for a single breeding season). After controlling for intercorrelations between the 10 nonredundant, sexually distinct traits using independent contrasts, we found a significant positive relationship between trait sex differences and

has an overall similar structure to male song and seems to be used similarly in countersinging, as well as mate interruption contexts. The use of female song in countersinging (see video at <https://vimeo.com/424642268>) just prior to clutch initiation is consistent with singing patterns and use of song for territory/nest defence or intrasexual competition in other northern temperate breeding species (Cain et al., 2015; Krieg &

Lin, & Lin, 2016; Sosa-López, Martínez Gómez, & Mennill, 2016; Wilkins, Seddon, & Safran, 2013, 2018), we know relatively little about female vocal signalling or its implications for broader evolutionary processes, even in classic study systems like barn swallows.

Overall Patterns of Sexual Differentiation in Signals

In contrast to a common assumption in the sexual selection literature, we found that overall patterns of sex differences are better explained by (surrogate measures of) selection on females, rather than selection for more elaborate males. That is, the overall magnitudes of sex-based differences in song form, tail streamer length and colour were predicted by how those traits correlated with female, but not male, reproductive performance. This does not mean that selection on males (i.e. through female choice) is not important or relevant in explaining sex differences. For example, as shown in Fig. 4b and Fig. A1, belly brightness – a known sexual signal in males within this population (Safran et al., 2016; Wilkins et al., 2015) – showed a strong negative association with genetic paternity (selecting for darker males with lower brightness). In turn, belly brightness in females showed a slight positive association with fledging success, and sex difference was low (0.8 SD), but significantly biased towards lighter females. That is, higher reproductive performance among darker males, combined with negligibly higher performance in lighter females correlates with an overall pattern of slightly darker males. In contrast, a strong positive correlation between element diversity and reproductive success in males and a strong negative correlation for females (i.e. divergent selection in the sexes) corresponds with a highly male-biased sex difference (–3.11 SD) towards more complex syllable composition of songs. While an additional analysis testing the combined impact

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A C

M.R.W. collected data, performed analysis and wrote the initial manuscript draft; K.J.O. measured and extracted acoustic metrics from audio files; L.B. undertook major restructuring of the manuscript; and R.J.S. provided project administration and resources for carrying out the work. All authors contributed significantly to manuscript writing and revision.

A

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A1 (continued)

Trait type	Trait	Definition
Visual	Tail length	Maximum length of two longest tail feathers (streamers), each of which was the average of 3 repeated measures (mm)
Visual	Brightness	The average percentage reflectance between 300 and 700 nm; lower values are darker; measured for throat, breast, belly and vent
Visual	Hue	The wavelength at the maximum slope of reflectance spectrum (in nm); low values pale/yellowish; high values dark/reddish; measured for throat, breast, belly and vent
Visual	Chroma	The proportion of light reflected in the red colour range (600–700 nm); higher values darker; measured for throat, breast, belly and vent

