Online Resource 1

Trill performance components vary with age, season, and motivation in the banded wren

Behavioral Ecology and Sociobiology

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A. Evaluation of time-of-day effects on the social context comparison

If there is a gradual increase or decrease in trill performance as a function of time of day, then our comparison of trill component changes between the two social contexts of dawn chorus versus playback challenges (Table 1c of main text) could be confounded, or, at worst, a spurious effect of time. We therefore coded the start time for each of the songs in this comparison to evaluate the role of time of day.

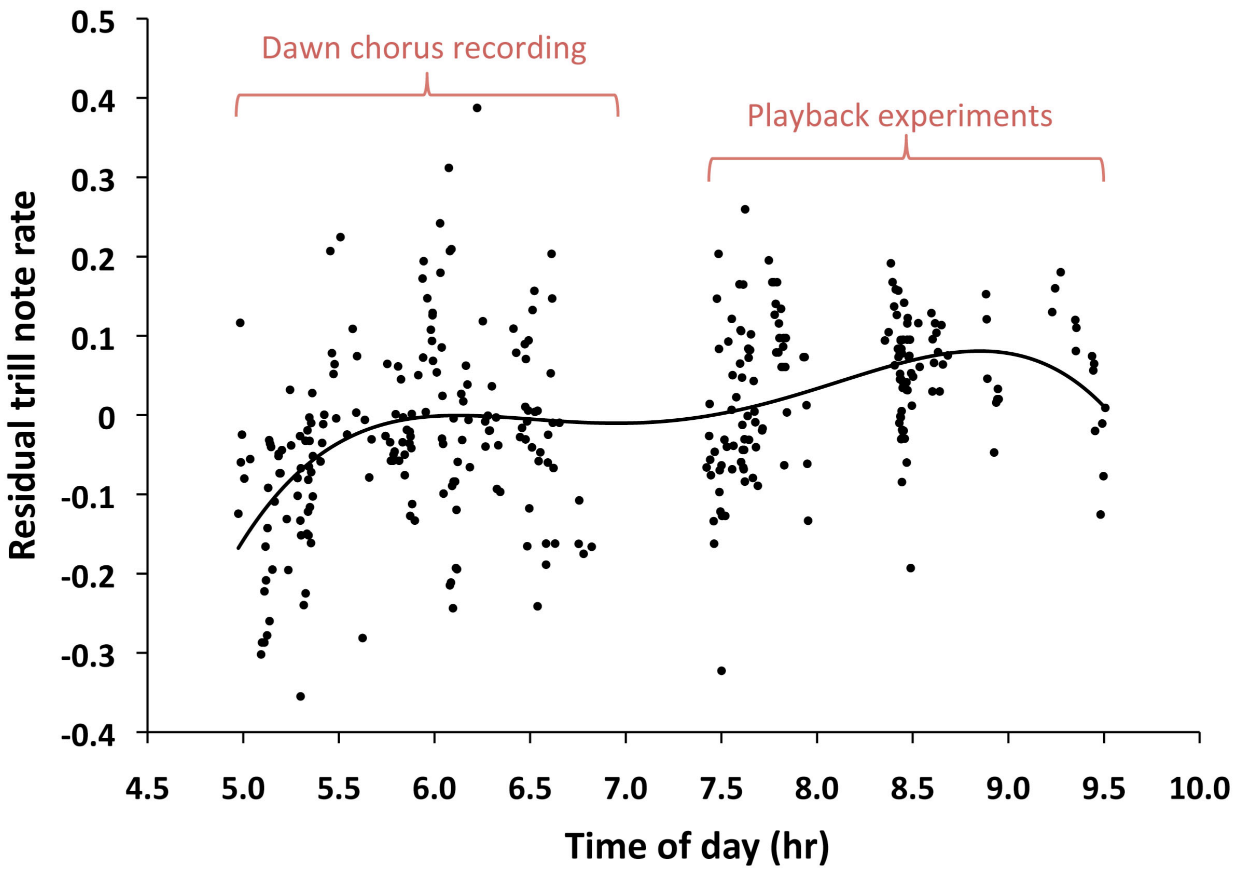

Figure S1. Trill note rate corrected for male and song type as a function of time of day for the social context dataset, fitted with a 4-degree polynomial regression. Dawn chorus recording extended from approximately 05:00 until 07:00, and playback experiments occurred between 07:30 and 09:30.

Figure S1 presents a plot of residual trill note rate (corrected for bird and song type differences) versus time of day. The increase in trill note rate with time of day is better explained by a 4-degree polynomial curve ($F_{4,345} = 23.18$, $p < 0.0001$, adj. $R^2 = 0.203$) than by a simple linear fit ($F_{1,348} = 23.18$, $p < 0.0001$, adj. $R^2 = 0.1551$). This plot shows that note rate rises rapidly during the first half hour of the morning, from approximately 05:00 to 05:30, and then levels off for the remainder of the dawn chorus period. During the playback period, there is an additional rise in note rate, which then falls off. Males first start singing in the dark before sunrise, and we have argued elsewhere (Burt and Vehrencamp 2005) that they song-type match each other intensely at this time without the risk of aggressive retaliation (chases and fights never occur during this period). The birds broadcast their songs very loudly (assessed by ear) from high perches around the edges of their territory, and it is possible that the slower trill note rate enables them to increase their trill note bandwidth and/or amplitude. As ambient light increases, the female mate usually appears, and the male may engage in countersinging interactions with her and with neighboring males. Trill note rate becomes higher but more variable, and amplitude may be lower. During territorial challenges simulated by playback, trill note rate increases even further, peaking around 08:30 as illustrated in Figure S1. As shown in Figure S2, the aggressive response to playback also peaks around this time.

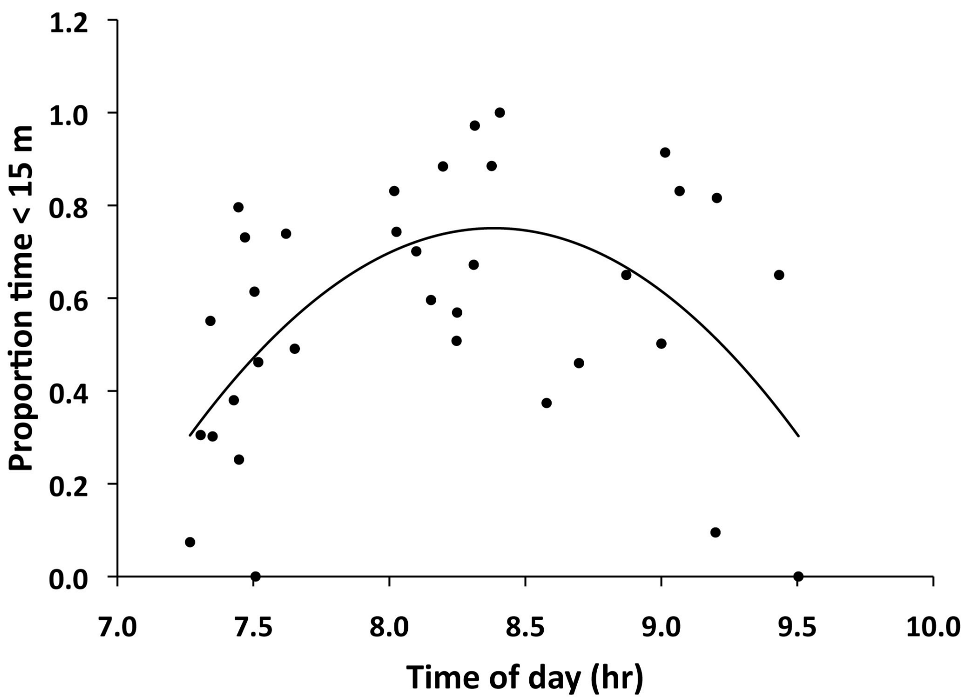

Figure S2. Aggressive response to playback, measured as proportion of time spent within 15 m of the speaker during and post playback, as a function of time for all of the playback experiments performed in 2005. Points were fitted with a 2-degree polynomial regression.

Changes in trill note rate during the morning therefore parallel the changes in social context, and in particular, the level of aggressive behavior displayed by the birds. Furthermore, our analysis of male and environmental variables associated with the magnitude of change in trill note rate also showed a tendency to increase when the playback stimulus contained the particular song type were had measured. This observation implies that when the playback matches the bird, his aggressive motivation is even higher. Note rate thus seems to be a very sensitive indicator of aggressive arousal or motivation.

Figure S3 shows trill frequency bandwidth as a function of time of day. Here we find a more gradual change, which could indicate that the bandwidth change shown in Table 1c is a time of day effect. A 4-degree polynomial fit ($F_{4,339} = 3.225$, $p < 0.026$, adj. $R^2 = 0.026$) is not significantly better than a linear fit ($F_{1,342} = 9.089$, $p < 0.003$, adj. $R^2 = 0.023$). The bandwidth measure is also potentially affected by the distance between singer and recordist, but we do not believe there was any systematic bias in distance between dawn chorus and playback recording. Moreover, bandwidth does not vary as a function of the intensity of aggressive response. Bandwidth is therefore unlikely to serve as an indicator of aggressive motivation in the same way that trill note rate does.

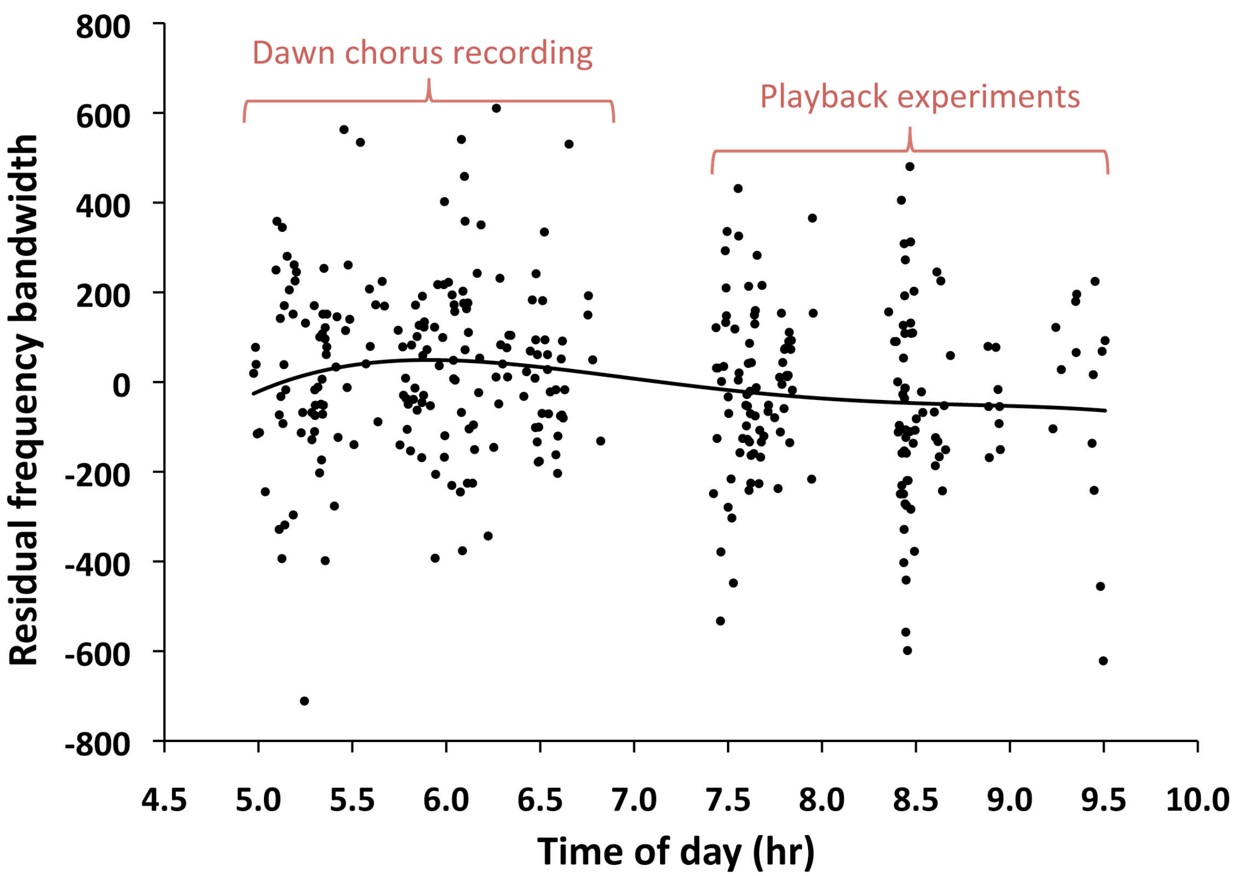

Figure S3. Trill note frequency bandwidth corrected for bird and song type as a function of time of day for the social context dataset, fitted with a 4-degree polynomial regression curve.

B. Trade-offs between trill performance components

Here, we evaluate within-male correlations between frequency bandwidth and note rate, and between vocal deviation and consistency, in the terminal trills of banded wren songs.

Trills consist of a series of frequency-modulated syllables. There is a physically constrained trade-off between the range of frequencies produced in each syllable (frequency bandwidth) and the rate at which the syllables are repeated (note rate). According to the source-filter theory of sound production, this relationship occurs because modulating the sound frequency requires the bird to change the tension on the syringeal membranes to produce the sweep in fundamental frequency (the source), and then to alter the volume of the upper vocal tract (oropharengeal cavity plus beak gape) so that the resonating chamber's dimensions match the fundamental frequency (the filter) (Goller and Suthers 1996; Goller and Larsen 1997; Hoese et al. 2000; Suthers and Zollinger 2004; Fletcher et al. 2006; Riede et al. 2006). Since a broader frequency sweep requires a larger change in the vocal tract, and the speed of motion is physically limited, a bird cannot simultaneously cover a broad frequency bandwidth and repeat the notes at a high rate (Podos et al. 2009). A plot of frequency bandwidth versus note rate for trilled songs in a variety of bird species or for different song types and individual males within a species yields a triangular distribution and an upper limit line. This plot for most banded wren song types can be seen in Figure 1 of Illes et al. (Illes et al. 2006). If within-male constraints follow the same trade-off principles as the between-male and between-song-type relationships, we would expect to find a negative correlation between bandwidth and note rate.

Consistent repetition of complex or broadband notes should also require a high level of skill. The movement of respiratory, syringeal, and vocal tract muscles must be precisely coordinated and integrated across several brain regions (Suthers et al. 1999; Suthers et al. 2002; Jarvis 2004; Ashmore et al. 2005; Sakata and Vehrencamp 2012; Mendez et al. 2012). In addition, the trill notes of banded wrens, like those of the northern cardinal (*Cardinalis cardinalis*), involve a transition between the low-register side of the syrinx and the high-register side; young birds show a clear gap between these two parts of the frequency sweep that becomes smoother with age (Suthers 1990; Botero and de Kort 2012). Producing a repeating series of these complex notes in exactly the same way requires precise coordination and practice. Moreover, it should be more difficult to repeat trill notes close to the performance limits with greater consistency than more easily produced notes (Botero and de Kort 2012). We therefore also predict a trade-off (in this case, a positive correlation) between vocal deviation and note consistency.

To statistically evaluate these trade-offs between frequency bandwidth and note rate, and between consistency and vocal deviation, we used within-subject centering to separate the within- and between-subject levels of correlation between two measured variables (van de Pol and Wright 2009). We pooled the data from all three datasets (age, season, and context) and then extracted the measurements for six common song types for separate analyses to eliminate the confounding effects of song type. For each song-type dataset, we computed a new variable with the mean note rate for each male (representing the between-male effect) and a second variable of the difference between each observed note rate and the male mean value (representing the centered, or within-male effect). We then ran a mixed model analysis with bandwidth as the dependent variable and mean bird note rate, centered bird note rate, and bird ID (random) as independent variables. The two fixed variables in this analysis independently test whether bandwidth varies between males as a function of individual differences in their mean note rate, and whether bandwidth varies within males as they vary their note rate for each song. A similar series of analyses were undertaken on each song type dataset to evaluate the between- and within-male correlation between consistency and vocal deviation, where consistency served as the dependent variable and mean vocal deviation rate, centered bird vocal deviation, and bird ID (random) were the independent variables. For simplicity we report only the results of the within-male correlations, since the between-male results varied greatly depending on idiosyncrasies of song-type variants among males. All statistical tests were run using JMP® Pro 9.0.2 (SAS Institute, Inc.).

Table S1 summarizes the within-male correlations between frequency bandwidth and note rate, and between trill note consistency and vocal deviation, for six song types. Bandwidth and note rate were negatively correlated in two of the six song types, consistent with the expected negative trade-off relationship. Trill note consistency and vocal deviation were positively correlated in three of the six song types (with a trend in the same direction in a fourth), suggesting a trade-off relationship between these two components.

Table S1. Within-male correlations between consistency and vocal deviation and between frequency bandwidth and note rate for six common song types. Statistical results (*F* and *p* values) are based on analysis of transformed variables, whereas slopes are based on analysis of untransformed variables for ease of comparison. Significant relationships are shown in bold.

	Bandwidth versus Note rate			Consistency versus Vocal deviation		
Song type	*F* (*df*)	*p*	Slope [a]	*F* (*df*)	*p*	Slope [a]
103sl	22.93 (1,372.3)	**<0.0001**	**−463.9**	2.753 (1,346.7)	0.098	+0.015
103fa	1.392 (1,116.0)	0.241	+209.9	0.324 (1,112.2)	0.570	−0.005
206sr	0.315 (1,387.9)	0.575	+56.1	5.358 (1,363.0)	**0.021**	**+0.013**
206cp	2.423 (1,207.1)	0.121	−190.2	10.04 (1,200.2)	**0.002**	**+0.016**
220a/b	3.412 (1,503.9)	0.065	+150.5	10.84 (1,485.5)	**0.001**	**+0.021**
220e	7.449 (1,122.0)	**0.007**	**−369.4**	0.565 (1,115.9)	0.454	−0.007

[a] Trill performance trade-offs predict positive correlations between consistency and vocal deviation and negative correlations between bandwidth and note rate.

Although frequency bandwidth and note rate changed in opposite directions in all three of our comparisons (see main text), this within-male analysis did not reveal as strong a trade-off as we expected. One possible explanation is that the note rate and bandwidth changes are small and the constraints are not apparent within this narrow range, as argued by DuBois et al. (2011).

Alternatively, perhaps only song types with trills closest to the performance limit are strongly subject to this trade-off. This hypothesis predicts a positive correlation between the slope of the bandwidth-note rate regression and the mean vocal deviation of each male's songs. We found no correlation between the slope and the mean vocal deviation ($r = 0.12$, $n = 72$, $p = 0.324$). Figure S4 shows the bandwidth-note rate plot of the common song types, with individual males and song types indicated by different symbols. The performance limit line was based on a larger sample of songs and types than the ones plotted here. A majority of males showed the predicted negative slope, including those with large vocal deviation means far below the performance limit. However, more than a third (10 of 27, 37%) of the males showed positive slopes. These males were all from the season comparison and increased their bandwidth as well as their note rate later in the season; most of the other birds in the season dataset reduced their bandwidth later in the season. What this graph seems to indicate is that most birds are operating below the performance limit line and therefore have a fair amount of leeway to vary both their bandwidth and note rate. This flexibility allows them to vary note rate and bandwidth in different social contexts.

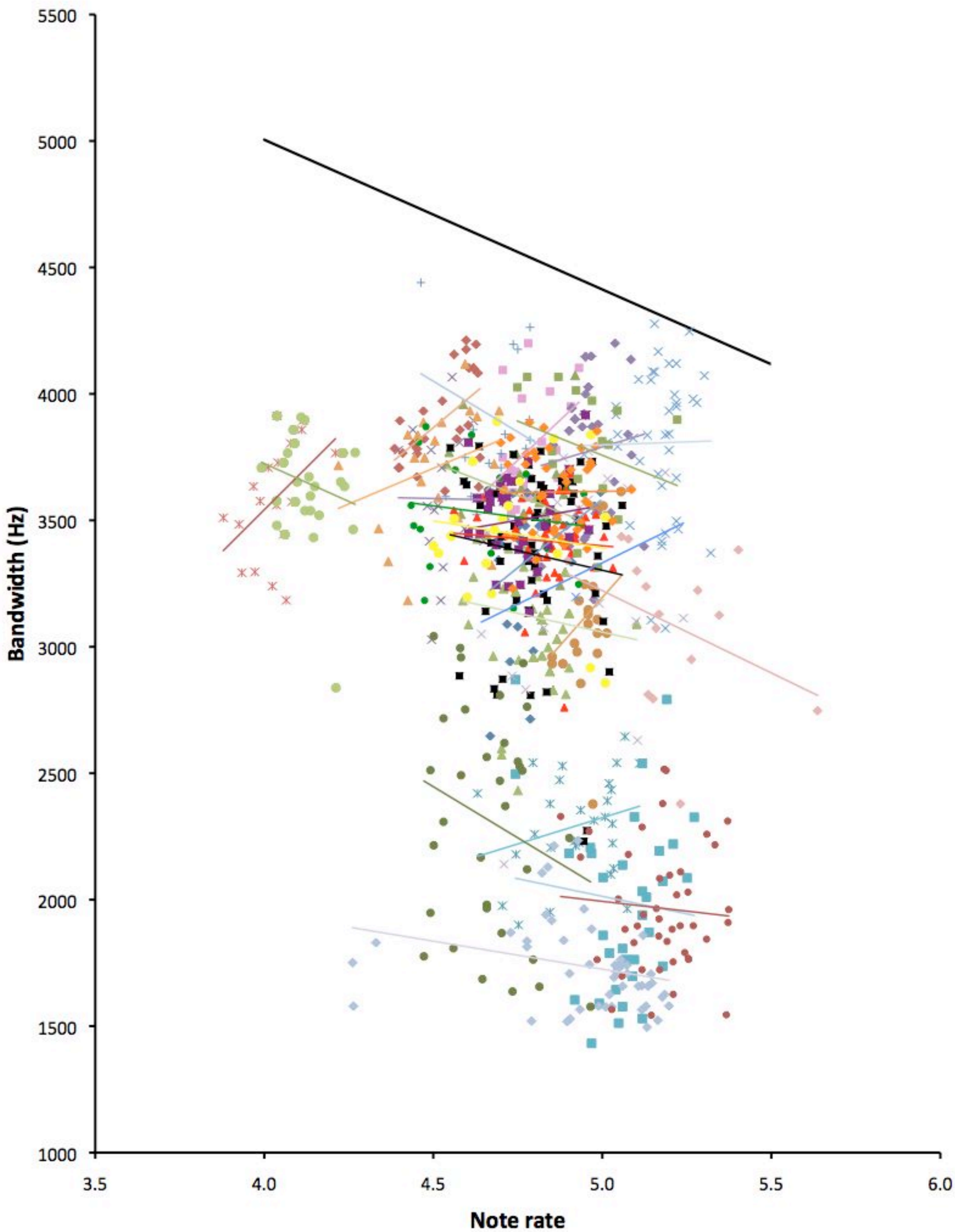

Figure S4. Trill bandwidth versus note rate, with different symbols and colors for subsets of males and song types. Trendlines for each subset are shown in matching colors. The heavy black line shows the performance limit for the banded wren, which was based on a larger sample of song types.

On the other hand, we did find a significant within-male trade-off between vocal deviation and note consistency across several song types. Given the strong selection to increase performance on both components (Illes et al. 2006; de Kort et al. 2009a,b; Cramer et al. 2011), it appears to be difficult for birds to perform trills that are both high in performance and consistent in delivery, as we would expect from a high-performance display. Nevertheless, we demonstrated here that individuals are able to increase both parameters as they age. Figure S5 shows a similar graph of individual male's slopes on the consistency versus vocal deviation plot. In this case, most of the males showed the expected positive slope, but 9 of the 29 males (31%) had a flat or negative slope. These cases came from either the age or seasonal comparisons in which the bird improved both vocal deviation and consistency.

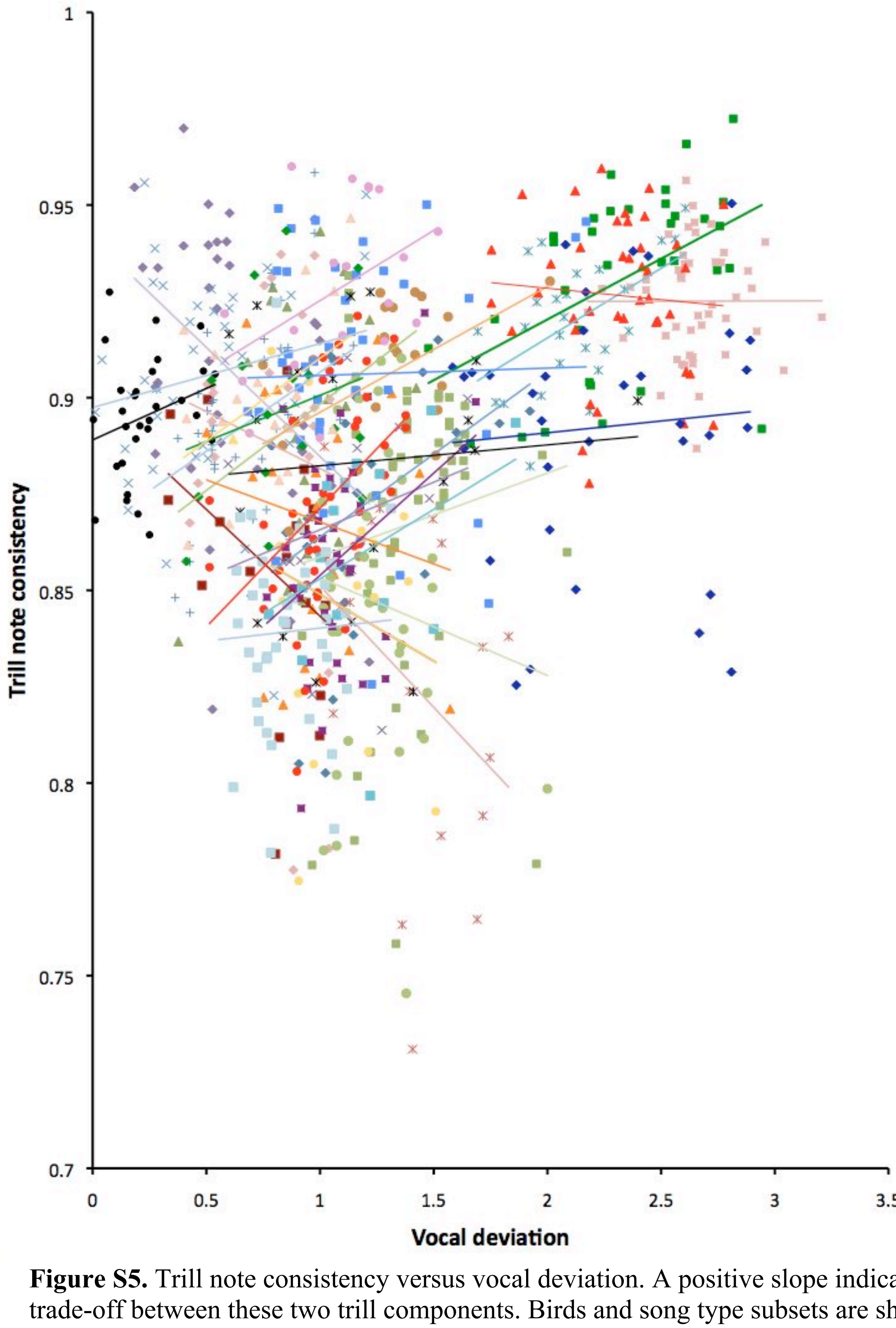

Figure S5. Trill note consistency versus vocal deviation. A positive slope indicates a trade-off between these two trill components. Birds and song type subsets are shown in different symbols and colors with matching color trendlines.

Another way to examine these trade-offs is to center the data on bird, song type, and state, which has the effect of assessing the presence of a trade-off from song to song within a single morning's recording session. This strategy also allows us to pool all song types. We found no within-state correlation between bandwidth and note rate ($F_{1,1649} = 0.024$, $p = 0.877$), but we found a positive within-state correlation between trill note consistency and vocal deviation ($F_{1,1557} = 25.54$, $p < 0.0001$). Figure S6 shows graphs of these two correlations based on state-centered data. Despite the large amount of scatter, the positive correlation between consistency and vocal deviation is highly significant and consist for most of the birds. This result implies that even within a day, an attempt by the bird to increase its performance (i.e., decrease the vocal deviation score) results in a reduction in trill consistency, and vice versa.

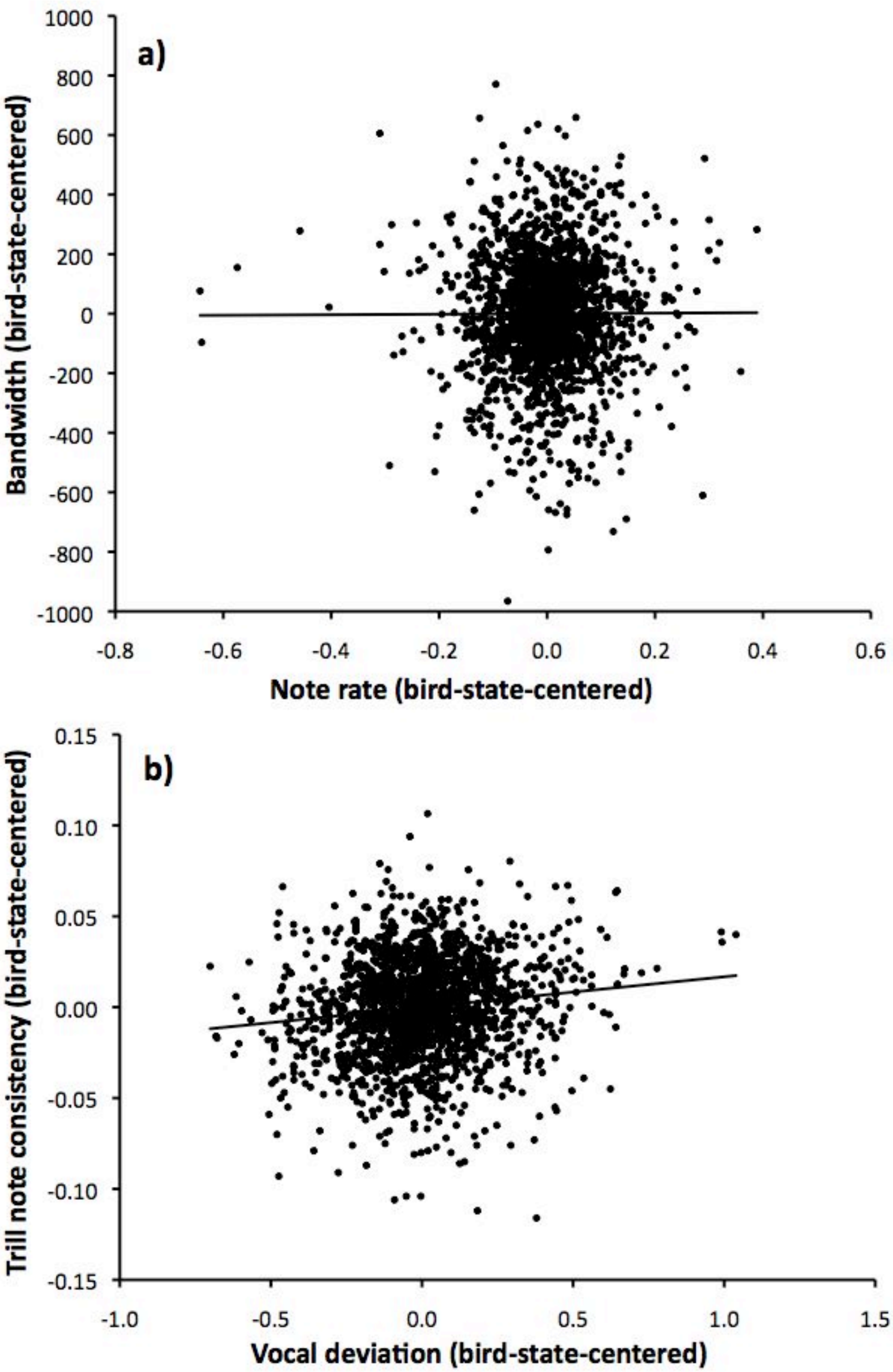

Figure S6. Within-bird-and-state correlations between (**a**) bandwidth and note rate and between (**b**) trill note consistency and vocal deviation. Graphs show the state-centered values for both axes, and the overall trendlines.

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