



To overlap or not to overlap: context-dependent coordinated singing in lekking long-billed hermits



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When using signals to attract mates or defend resources, animals often overlap the voices of other individuals in close proximity. In such contexts signal masking is likely and animals would benefit by adopting behavioural strategies that modify the timing of signals to minimize the negative effects of masking or take advantage of its signalling value. Indeed, temporal coordination has been commonly described in a wide variety of taxa, but compelling evidence demonstrating that it arises as an active process is scarce. Here we investigate the degree of coordinated singing by lekking long-billed hermit hummingbirds, *Phaethornis longirostris*, in the Neotropics, using randomization tests to study the timing of vocal signals. We first demonstrate that a randomization statistical approach is robust at detecting coordinated singing in simulated data. Then, we show that long-billed hermits engage in vocal interactions in which either song alternation or song overlap is used. Furthermore, we show that singing behaviour varies with the distance between singers: hermits alternate their songs when they are in close proximity, and they overlap songs at farther distances. Birds achieve these two behaviours by modifying the time intervals between signals. The association between pattern of coordination and distance is not fully explained by any of the current hypotheses for song overlap and suggests that multiple context-dependent singing strategies could be involved. Our findings provide compelling evidence on vocal coordination as an active process in this species and validate an analytical approach that could be extended to investigate similar patterns in other taxa.

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When using signals to attract mates or defend resources, individuals often broadcast their signals in close proximity to other signallers. In such contexts, animals routinely modify the structure or timing of their signals compared to when they are delivered in isolation (Bradbury & Vehrencamp, 2011). Coordination of acoustic signals in the form of temporal alternation or overlapping has been documented across a wide variety of taxa, including vocal duets in primates (Haimoff, 1986), choruses of mating aggregations in anurans, insects (Greenfield, 1994) and fish (McCauley & Cato, 2000), drumming in spiders (Kotiaho, Alatalo, Mappes, & Parri, 2004) and vocal interactions in whales (Schulz, Whitehead, Gero, & Rendell, 2008) and birds (Mennill &

Ratcliffe, 2004). Several hypotheses have been presented to explain these behaviours (reviewed in: Greenfield, 1994; Helfer & Osiejuk, 2015). When overlapping in time, signals could mask each other, affecting the ability of receivers to detect the signals. By alternating their signals, animals could prevent degradation due to masking (interference avoidance hypothesis; Ficken, Ficken, & Hailman, 1974; Wilson, Ratcliffe, & Mennill, 2016). By overlapping their signals, on the other hand, animals may amplify their combined signals and communicate across greater distances (signal enhancement hypothesis; Bates et al., 2010; Rehberg-Besler, Doucet, & Mennill, in press; but see Bradbury, 1981). Animals may also use overlapping as a proximity signal of aggressive intent, for example, when animals are more likely to engage in agonistic interactions when overlapping an opponent's songs (aggressive signalling hypothesis; Kunc, Amrhein, & Naguib, 2006) or as a signal of submission used to de-escalate aggressive interactions (submissive signalling hypothesis; Searcy & Beecher,

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2009). Both alternation and overlapping could also evolve as a response to a female sensory bias towards leading signals as in some chorusing insects (precedence effect hypothesis; Greenfield, Tourtellot, & Snedden, 1997). Alternatively, coordination could constitute a signal by itself, as when it provides information on the relative competitive ability of a coalition of signalling individuals (coalition quality hypothesis; Hall & Magrath, 2007). Nevertheless, compelling evidence demonstrating that the described patterns arise as an active process is relatively scarce, mostly due to the lack of appropriate analytical tools (Helfer & Osiejuk, 2015; Masco, Allesina, Mennill, & Pruett-Jones, 2015).

Despite apparently being a common phenomenon, distinguishing vocal coordination (i.e. alternation or overlapping) from patterns arising by chance has proven to be challenging using conventional statistical approaches. Null model randomization techniques have been proposed as an alternative approach for studying vocal coordination (Helfer & Osiejuk, 2015; Maynard, Ward, Doucet, & Mennill, 2012) and recently have been applied to vocal signals in songbirds (Masco et al., 2015; Maynard et al., 2012; Yang, Ma, & Slabbekoorn, 2014). However, the utility of null model randomization for detecting specific temporal patterns in vocal interactions has, to our knowledge, not been validated by using data that conforms to a priori patterns (e.g. overlapping or alternating signals). Such an approach is commonly used in other biology research fields (e.g. species distribution modelling; Broennimann, 2012; molecular ecology: Anderson, Skaug, & Barshis, 2014; comparative phylogenetic methods: Kaliontzopoulou & Adams, 2016) and provides a powerful tool for establishing the validity of randomization methods.

Leks of highly vocal species are usually crowded acoustic environments with many individuals singing simultaneously while competing for mating opportunities. In such a context any signalling strategies that would help males attract female attention would be strongly favoured. Hence, leks provide an ideal system in which to assess behavioural strategies associated with coordinated singing (Maynard et al., 2012). In this study we investigate singing interactions between lekking long-billed hermits, *Phaethornis longirostris*. Males of this species spend most of the day singing in closely packed territories within a lek (average \pm SD distance between territory centroids: 39.2 ± 26.5 m), and therefore many individuals share the same active acoustic space (i.e. the distance over which the song is detected by conspecifics). It has been suggested that neighbouring male hermits singing at the same time alternate their songs by modifying their singing rate (Stiles & Wolf, 1979), although this has not been formally tested. Here we test this hypothesis by examining natural patterns of simultaneous singing using microphone arrays (hereafter, we use the phrase 'simultaneous singing' to refer to animals singing at the same time but not necessarily in a coordinated manner). We specifically evaluate (1) the coordination in simultaneous singing bouts compared to a null model using a randomization approach, (2) the association between vocal interactions and spatial proximity of the singers and (3) the modifications in singing behaviour involved in achieving coordination.

METHODS

We conducted field work at four leks located in La Selva Biological Station, Costa Rica ($10^{\circ}23'N$, $84^{\circ}1'W$) between January and May 2012. Birds were mist-netted at the lek area and marked with foam tags that had unique three-colour combinations, attached to the back of the bird with nontoxic eyelash glue, LashGrip-Ardell® (Ardell International, Los Angeles, CA, U.S.A.; Baltosser, 1978; Gonzalez & Ornelas, 2009; Kapoor, 2012; Stiles & Wolf, 1973). We

observed singing behaviour and physical interactions at the leks to distinguish territorial males from females and nonterritorial males, to locate these males' display perches and, based on these perches, to map the location and extent of the males' territories.

Microphone Array

We deployed six automated recording devices (model: Song-Meter SM2+GPS, Wildlife Acoustics Inc., Concord, MA, U.S.A.) with an integrated GPS device to synchronize the recordings (Mennill, Battiston, Wilson, Foote, & Doucet, 2012). Each device recorded from two omnidirectional microphones (model: SMX-II; Wildlife Acoustics Inc.) with a sensitivity of -35 dBV/Pa and frequency response of 20–20 000 Hz. A maximum distance of 20 m was used for spacing adjacent recorders (mean \pm SD distance between adjacent recorders: 16.03 ± 7.3 m). Recorders were placed at a height of approximately 2 m above the ground, close to the average \pm SD perching height of lekking males (2.15 ± 1.31 m). We attached the recorders to trees not used as perches by territorial males. Recorders were programmed to sample for 29 min at 30 min intervals between 0530 and 1730 hours (1 min pauses provided the recorders with sufficient time to store the recordings to SD cards). Recordings were made with a two-channel lossless compressed WAC format at a sampling rate of 44.1 kHz and an accuracy of 16 bits; we converted recordings to WAV format for subsequent analyses.

We positioned the recorders in locations that fell in between individuals' territories, to maximize the opportunity to record multiple males. The position of perches and recorders were mapped with a laser range finder and a compass using a 20×20 m grid system as reference (estimated accuracy: ± 1.0 m). The relative position of the singing males was used to determine their identity (described below). The microphone array was deployed 1 day after mapping the territories in each lek to decrease the risk of birds shifting their territorial position. In three leks, two separate array configurations were needed to cover all territories within each lek (i.e. half of the lek was recorded for a period, and the other half during a subsequent period). In each configuration, we recorded birds with the microphone array over a 6-day period, the longest time allowed by the available battery power.

Analytical Approach

We selected simultaneous singing bouts involving two or more animals (hereafter 'bouts') by visually inspecting all 12 channels (6 recorders \times 2 channels) from the recording sessions using the software Syrinx-PC (J. Burt, Seattle, WA, U.S.A.). Long-billed hermit songs were by far the most common sound in these recordings, and were easily distinguishable from any other sounds based on frequency range (2–9 kHz) and distinctive spectrographic pattern (Araya-Salas & Wright, 2013). The start and end of bouts, including any solo singing before and/or after the bout, were used to delimit discrete bouts in further analyses. Bouts in which more than two individuals were found to be singing were excluded (11% of bouts involved more than two animals), allowing us to focus on the most common type of bout.

The identity of singing males was established based on the distance between lek territories and the recorders in which songs showed the highest amplitude. Birds are faithful to the perches within their territories and territory locations remained stable during our usual observation period (1–2 weeks; Stiles & Wolf, 1979). We placed the recorders in between territories in such a manner that we could assume the bird that was recorded was in fact the owner of the territory. However, the spatial configuration of territories made it difficult to identify some birds in this way. In

those cases we created maps with the position of singing perches and recorders to visually determine the most likely singing male. In addition, the song type structure of the singing males and the presence of songs from close neighbours in other channels were used to confirm the birds' identities. Several distinctive song types can occur within a lek, and each song type is usually shared by subgroups of lekking individuals (Araya-Salas & Wright, 2013). Bouts were assigned to a given individual only when we were able to discard all other candidates based on mismatches between singing location and singer song type (25% of the birds recorded were labelled as unknown). This method could potentially bias the sample in which birds were identified by preferentially excluding bouts from birds with shared song types in close proximity, because identification based on location of territories is more difficult in these cases and different song types cannot be used to identify individuals. However, bouts at close distance involving the same song types represented a similar portion of the data for both identified and unidentified birds (23.5% and 29.6%, respectively).

The start and end of individual songs within bouts was determined either manually on Raven Pro 1.4 (Charif, Waack, & Strickman, 2010) or automatically by detecting songs based on cross-correlation of a manually selected template signal from the same sound file. Automatic detection was conducted with the R package 'monitor' (Hafner & Katz, 2015). Automatic detections were visually checked and corrected when necessary using the R packages 'tuneR' (Ligges, Krey, Mersmann, & Schnackenberg, 2014), 'seewave' (Sueur, Aubin, & Simonis, 2008) and 'warbleR' (Araya-Salas & Smith-Vidaurre, 2016).

Statistical Methods

We used a Monte Carlo randomization test in the R package 'warbleR' (Araya-Salas & Smith-Vidaurre, 2016) to evaluate the statistical significance of the number of times in which a bird began singing before its counterpart had completed its song (hereafter 'song overlaps'). The test calculated the probability of finding an equal or more extreme number of overlaps in bouts. Sequences of songs and the silent intervals between signals (variously termed 'song delay' or 'gaps' in the literature, hereafter 'gaps') of each individual were shuffled many times to produce a null distribution of the number of overlaps expected if signal timing of males' songs within a bout were independent of each other. In this randomization procedure, each song was separated from the subsequent song by a gap, which produces the most biologically realistic singing bouts among other currently available methods (reviewed in Masco et al., 2015). The *P* value was calculated as the proportion of the random number of overlaps that were equal to or more extreme than the observed value. Although this test is equivalent to the 'KeepGaps' methods described in Masco et al. (2015), we developed this technique for current analyses independently of Masco et al. (2015), as the software described in Masco et al. (2015) was not available when we began our analyses. Significant singing coordination patterns were considered as the *P* values at both tails of the null distribution (alternated singing: $P < 0.025$; overlapped singing: $P > 0.975$; expecting 5% of significantly coordinated bouts to arise by chance at $\alpha = 0.05$). Individual tests were calculated for each bout. All randomization tests were iterated 10 000 times. This randomization procedure was applied to both real and simulated data (described below). For the real data, we ran this analysis for both the complete data set, including bouts with unidentified singers, and for the subset of bouts in which bird identity for both singers was known (hereafter 'known dyads'). For the data set consisting of known dyads, we used a chi-square test to evaluate whether the number of coordinated bouts per dyad deviated from chance (expecting 5% of significantly coordinated bouts).

We evaluated the performance of the randomization test in detecting patterns of simultaneous singing bouts. Simulated bouts were generated using the mean song duration from our sample of long-billed hermit bouts. Song duration was allowed to vary within the mean standard deviation across song types derived from the same data. We simulated gap duration by randomly selecting them from the pool of observed gaps. All simulated bouts started with a gap. Overlapping singing was simulated by generating one song sequence and then creating a second sequence with a 75% chance of starting the song after the beginning but before the end of a song in the counterpart sequence (i.e. overlap). Alternating singing was simulated by creating a second sequence with a 25% chance of starting the song after the end but before the beginning of its counterpart songs (i.e. a 75% probability of alternating, the same probability of overlap in overlapping simulated bouts). Hence, not all songs were overlapped or alternated, as is the case during natural bouts of long-billed hermit interactions. Finally, uncoordinated bouts were simulated by simply joining two independently generated song sequences. We simulated 1000 bouts for each of the three patterns and calculated the *P* value from the randomization test (10 000 iterations). The duration of all simulated bouts was equal to the mean duration of long-billed hermit bouts.

To further understand the dynamics of the vocal interactions, we evaluated whether patterns of overlap or alternation were associated with social or contextual factors (described below). We calculated the proportional difference between the observed and expected number of overlaps by chance according to our randomization procedure $((\text{obs} - \text{exp})/\text{exp})$ as a measure of singing coordination performance (hereafter 'coordination score'). Positive coordination scores imply a tendency to overlap songs, whereas negative scores are associated with song alternation. We assessed the effect of song-type sharing (singing individuals having the same song type, or not), time of the day, duration of the bout and physical distance between the singing individuals. Song-type sharing was determined by visual inspection of spectrograms, a method that has shown high consistency in this species (Araya-Salas & Wright, 2013).

We found that microphone array-based triangulation of animal positions using established techniques (Mennill et al., 2012) did not work effectively for long-billed hermits because of the brevity and low amplitude of their songs. We used a different approach, where we estimated the distance between two singing males as the distance between the two recorders that they were closest to. The distance between the recorders is expected to be related to the actual distance between the birds. Given the higher uncertainty of this alternative method, we used the two extremes of the distribution of the distance between the two recorders across all of the bouts that were analysed (25% and 75% quartiles) to classify bouts into two distance categories (close: 0–13.4 m; far: 27.0–75.6 m). From all possible dyads in a lek, 34% (range 3–57%) of bouts were sung within the close range and 87% (range 57–100%) were sung within the far range. Bouts in the interquartile range were excluded from analysis in which distance was involved.

Finally, we considered whether birds modify the gaps between songs when engaging in singing coordination. The onset of singing is typically not synchronized; one of the birds has already sung several songs (solo singing) before the second bird has begun singing. We evaluated whether average gap duration and its variation (coefficient of variation; hereafter 'gap variation') changed in these two singing modes (solo singing or simultaneous singing). Only bouts with at least 10 song repetitions during solo singing were analysed. Territorial males usually engage in interactive visual displays with intruders or females (Stiles & Wolf, 1979), which requires a pause in singing activity. Therefore, we excluded unusually

long gaps by removing the longest 5% of the gaps (this was done separately for solo and simultaneous singing).

Statistical Analysis

Model selection on linear mixed-effect models fitted with maximum likelihood was used to test the effect of context on the coordination score. This approach was also used to evaluate the effect of coordination pattern (alternated, overlapped, or uncoordinated) and singing mode (solo versus simultaneous singing) in gap duration and gap variation. We used Akaike Information Criteria corrected for small sample sizes (AICc) as a measure of model relative support, given the small sample size in relation to the number of estimated parameters. A null model of no effect was also included in each selection routine. The best models were selected as the lowest AICc models that accounted for at least 95% of the AICc weights in the candidate set. On this subset we calculated model-averaged effect sizes with a 95% confidence interval for each fixed effect. Effect sizes that did not overlap with zero were considered to have an effect on the response variable. Models were produced for both the complete data set and the subset from known dyads. Analyses on the subset of known dyads were run to control for pseudoreplication, while analyses on the complete data set aimed to confirm the results based on a larger, more robust, set of recordings. We also included songs from identified singers in bouts in which only one bird was identified for analyses on gap duration and variation (hereafter 'known individuals'). The analyses were run using the R packages 'nlme' (Pinheiro, Bates, DebRoy, Sarkar, & RCore Team, 2016) and 'AICcmodavg' (Mazerolle, 2016).

For models with coordination score as the response variable, we included time of the day, duration of simultaneous singing, song-type sharing (categorical: shared or unshared) and distance (categorical: close or far) as fixed effects and lek identity as a random effect. Time of day, duration of simultaneous singing and song-type sharing were included as they influence the structure (song type; Araya-Salas & Wright, 2013) and activity pattern (time of day, bout duration; Stiles & Wolf, 1979) of vocal behaviour in long-billed hermits, potentially affecting the likelihood of engaging in coordinated singing. We also included dyad and lek identities as random effects when analysing data from known dyads. Individuals were involved in an average of 1.73 dyads (range 1–4). We analysed on average 6.36 bouts per dyad (range 1–20). We included coordination pattern and singing mode as fixed effects for models in which gap duration or gap variation was the response variable. We also included bout as a random effect for the complete data set and bout nested within individual as a random effect for data from known individuals.

Ethical Note

All activities described were reviewed and authorized by the Institutional Animal Care and Use Committee at the New Mexico State University (IACUC-2011–020) and were performed under the research permits 152-2009-SINAC and 063-2011-SINAC from Costa Rican Authorities. Prior to the study, all subjects were captured via mist nets and handled briefly for morphometric measurement and marking with small coloured tags before being released at site of capture. During the study itself the birds behaved naturally without interference from the investigators.

RESULTS

During natural bouts of song recorded with microphone arrays, long-billed hermits alternated songs at above-chance levels in 43 bouts (29 for known dyads) and overlapped songs at above-chance

levels in 38 bouts (27 for known dyads; Figs. 1a and 2). Together, overlapping and alternating bouts represented 44% of the 185 bouts analysed (46% of the 123 bouts from known dyads); the remainder were uncoordinated singing. Most dyads engaged in vocal coordination (either overlapping or alternating): 73% of the dyads had at least one singing bout with a significant coordination pattern; 47% of them produced alternating bouts and 52% overlapping bouts. The number of coordinated bouts per dyad was significantly higher than expected by chance ($\chi^2_{18} = 42.2$, $P = 0.001$). Bouts lasted, on average, 66.9 s (range 5.1–438.7) with an average of 220 songs (range 24–1315). Despite many simultaneous singing bouts presenting significant vocal coordination, none showed perfect synchronization throughout the entire period of simultaneous singing (i.e. with all songs being perfectly alternated or overlapped).

We used simulated bouts of song to assess whether the randomization technique could correctly identify whether interactions involved overlapping, alternating or uncoordinated singing. The randomization test correctly detected the coordination pattern in 97.4% of the 3000 simulated bouts, with 99.9% of the simulated overlapping bouts ($P > 0.975$), 96.9% of the alternating bouts ($P < 0.025$) and 95.6% of the uncoordinated bouts ($0.025 < P > 0.975$) correctly detected. From the 4.4% of bouts that were uncoordinated and classified incorrectly (type I error), 2.7% showed an overlapping pattern and 1.7% an alternating one.

Both the distance between singers and the bout duration showed a significant effect in our mixed-effect models on the complete data set. Coordination score was negative (i.e. alternation of songs) when individuals were in close proximity, and it was positive (i.e. overlap of songs) when individuals were farther away (Fig. 1b, Table 1). Bout duration had a small effect on coordination score, with long bouts associated with negative coordination scores (Table 1). Time of day and song-type sharing did not have any detectable effect (Fig. 1c, Table 1). The four best mixed-effects models fitted for coordination score ($N = 61$ bouts) included distance as a predictor (Supplementary Table S1). Nine of 12 alternating bouts identified by our randomization test occurred at close distances, while all 11 overlapping bouts occurred at far distances.

When looking at the subset of bouts from known dyads (34 bouts, 12 known dyads), all four of the best models included distance as a predictor (Supplementary Table S1). Once again, coordination score was negative between individuals in close proximity but positive at far distances (Fig. 1b). Song-type sharing was included in two of the models, whereas bout duration and time of day were each included in a single model. However, no significant effect was found for any of these variables (Fig. 1c, Table 1). Four of six alternating bouts in this data set occurred at close distances, while all five overlapping bouts occurred at far distances.

Gap duration was best explained by a single model that included the interaction between singing mode (solo or simultaneous singing) and coordination pattern (alternation or overlap) for the complete data set ($N = 67$ bouts; Table 1). Gaps were longer during portions of bouts that included simultaneous singing compared to solo singing for both alternating and uncoordinated bouts, but not during overlapping bouts (Fig. 1d, Table 1). Furthermore, gaps in simultaneous singing portions of bouts were longer during alternation compared to gaps in uncoordinated and overlapping bouts (Table 1). We did not observe any differences in gaps during solo singing between any of the three coordination patterns (Table 1). A similar pattern emerged when analysing the data from known individuals (51 bouts, 12 individuals). The best two models included both singing mode (i.e. solo or simultaneous singing) as the single predictor and an interaction between singing mode and coordination pattern (Supplementary Table S1). Gaps were also longer during simultaneous singing for alternating and uncoordinated bouts, and did not differ between solo singing that preceded bouts

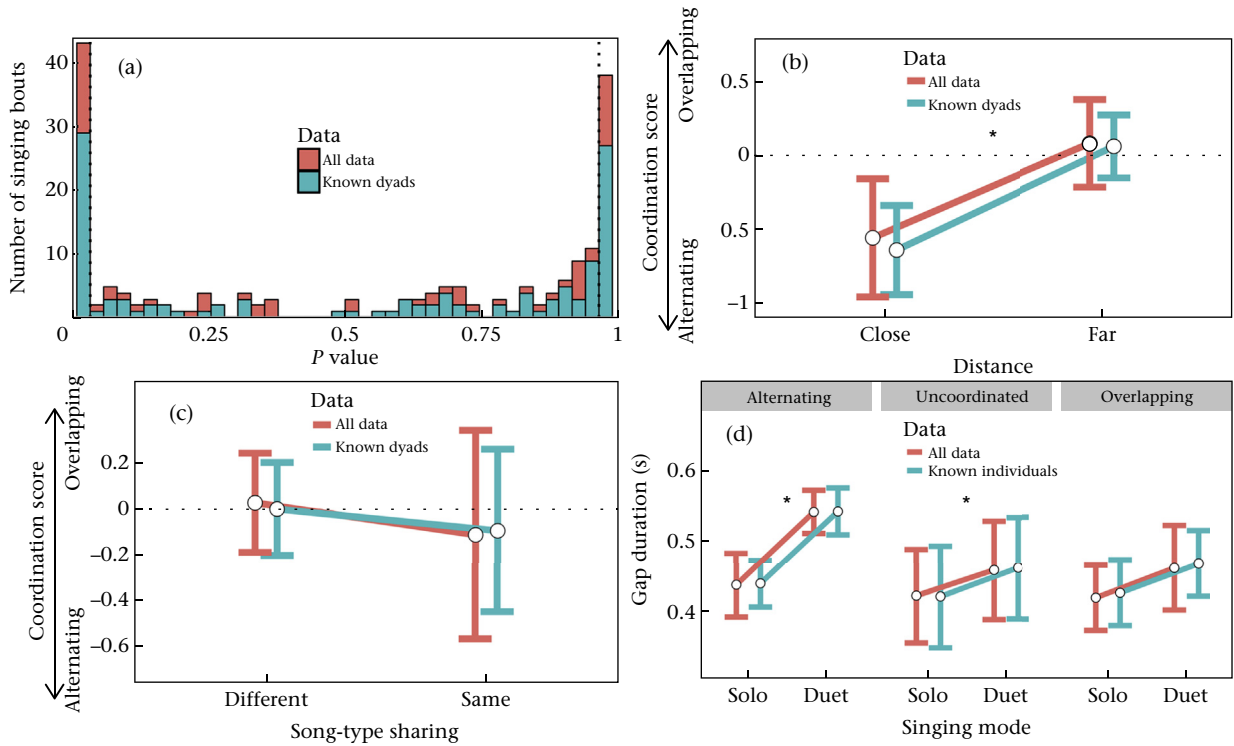


Figure 1. (a) Overlaid histograms of the *P* values from tests to detect nonrandom singing patterns in simultaneous bouts of song by male long-billed hermits. Dotted lines delimit the cutoff *P* values used to determine singing alternation ($P < 0.025$) or singing overlap ($P > 0.975$). (b) Coordination score by distance categories showing significant tendencies for alternation of song when singing dyads were in close proximity (<13.4 m) and for overlap of song when singing dyads were far apart (>27 m). (c) Coordination score by song-type sharing demonstrating a lack of difference in coordination between dyads of singers with either the same or different song types. (d) Gap duration by singing mode and coordination pattern in solo (Solo) and simultaneous (Simul) singing. Coordination score = (observed overlaps – expected overlaps)/expected overlaps. In all graphs results are shown for the complete data set (all data; a: $N = 185$ bouts; b–c: $N = 61$ bouts; d: $N = 67$ bouts) and the subset from known individuals/known dyads (a: $N = 121$; b–c: $N = 34$ bouts; d: $N = 51$ bouts). In (b)–(d), white circles represent means and error bars represent standard deviations. Asterisks show effect size 95% confidence intervals that did not overlap with zero. Horizontal dotted lines in (b) and (c) represent the expected score expected by chance. ‘Known dyads’ refer to singing bouts in which the identity of both singers was determined; ‘all data’ includes both ‘known dyads’ bouts and bouts from unidentified singers.

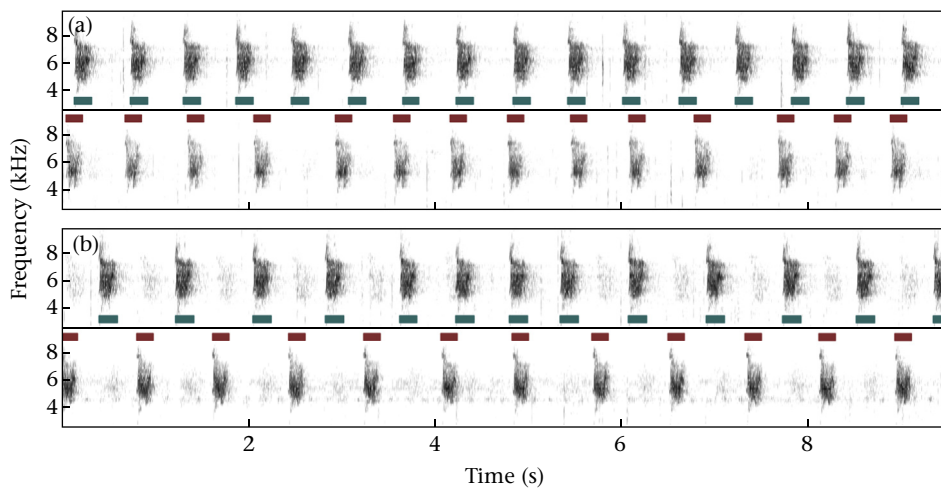


Figure 2. Spectrograms of real bouts of simultaneous singing by long-billed hermits classified as (a) overlapping or (b) alternating. Line segments correspond to the start and end of signals, illustrating the amount of song overlap. Spectrograms created in the R package ‘seewave’ (Sueur et al., 2008).

with either of the coordination patterns (Fig. 1d, Table 1). Gaps in bouts of simultaneous singing were longer during song alternation than in uncoordinated bouts but did not differ from gaps in overlapping bouts (Table 1).

The best two models for gap variation also included singing mode and the interaction between singing mode and coordination

pattern for the complete data set ($N = 67$ bouts; Supplementary Table S1). Gaps were more variable during simultaneous singing portions of bouts compared to solo singing portions in all coordination patterns (Table 1). Qualitatively equivalent results were obtained from the analysis of song bouts from known individuals (51 bouts, 12 individuals; Supplementary Table S1).

Table 1
Effect sizes and 95% confidence intervals for the fixed effects that significantly predicted coordination score and gap duration and variation

Response variable	Predictor	Effect size	Lower 95% CI	Upper 95% CI
Coordination score				
All data	Distance	0.556	0.321	0.791
	Duration	−0.002	−0.003	0.000
Known dyads	Distance	0.508	0.271	0.745
Gap duration				
All data	Singing mode (in alternating events)	0.106	0.063	0.148
	Singing mode (in uncoordinated events)	0.040	0.011	0.069
	Overlapping vs alternating (in simultaneous singing)	0.080	0.036	0.125
	Alternating vs uncoordinated (in simultaneous singing)	0.084	0.048	0.120
	Known individuals	Singing mode (in alternating events)	0.087	0.028
Known individuals	Singing mode (in uncoordinated events)	0.048	0.010	0.086
	Alternating vs uncoordinated (in simultaneous singing)	0.067	0.001	0.134
Gap variation				
All data	Singing mode (in alternating events)	0.040	0.014	0.066
	Singing mode (in uncoordinated events)	0.042	0.020	0.063
	Singing mode (in overlapping events)	0.043	0.016	0.071
Known individuals	Singing mode (in alternating events)	0.040	0.015	0.066
	Singing mode (in uncoordinated events)	0.042	0.020	0.063
	Singing mode (in overlapping events)	0.043	0.016	0.070

Effect sizes on gap duration are shown by level combinations of singing mode (solo and simultaneous singing) and coordination pattern (alternating, overlapping or uncoordinated). [Supplementary Table S2](#) includes the effect sizes for nonsignificant factors.

DISCUSSION

In this study we evaluated whether simultaneous singing in long-billed hermits occurs in a coordinated manner and, if so, whether coordination is associated with a modification in singing behaviour. We found that the birds often sing in a coordinated manner: during vocal interactions, both song alternation and overlap are common. The particular coordination strategy is related to the distance between singers; animals alternate when they are in close proximity and overlap at greater distances. Coordination is also associated with an increase in both the time intervals between subsequent songs and the variation of those intervals, indicating that an active change in singing behaviour is used to achieve coordination. Overall, our findings provide strong evidence that song coordination arises as an active process, whereby individuals modify the timing of their singing when engaging in simultaneous signalling. In addition, coordinated singing seems to be a context-dependent strategy in which the coordination pattern adopted is linked to the distance between interacting individuals.

We evaluated our method for detecting vocal interactions by testing its performance on simulated bouts. The method was robust at detecting overlapping and alternating bouts and did not show biases for mistakenly classified uncoordinated bouts. The method had an acceptable type I error (4.4%) for detecting uncoordinated bouts. Hence, it seems unlikely that the high occurrence of vocal coordination observed in actual bouts of simultaneous singing by long-billed hermits arose as an artefact of our statistical method. Long-billed hermits tend to reduce their singing rate at the end of

solo bouts (Stiles & Wolf, 1979), which could affect the randomization procedure as it disrupts any bout-level patterning. However, songs in simultaneous singing bouts do not usually end at the same time; one bird remains singing while the other one stops. This means that in most cases the slow-rate ending of individual singing bouts do not overlap in time when singing interactive bouts (in case the slow-rate ending occurs in simultaneous bouts). In addition, the randomization procedure takes into account the gap duration for estimating number of overlaps expected by chance. Hence, it is unlikely that this could have affected the performance of the test. Overall, this approach provides a compelling tool for assessing singing coordination and supports the utility of randomization tests. However, further testing across a range of bout and song durations is warranted for fully assessing the robustness of this tool.

The ‘incidental phenomenon hypothesis’ proposes that signal overlap is an unintended phenomenon, and under this hypothesis overlapping is not expected to occur more often than chance (Helfer & Osiejuk, 2015; Searcy & Beecher, 2009, 2011). Alternatively, coordination could be the result of individuals actively attempting to modulate the timing of their signals according to the timing of their counterparts. Our study provides compelling evidence that coordination in long-billed hermits does not arise randomly, but rather by the active behavioural strategy of the individuals engaged. Interacting birds achieved coordination by modifying the length of intervals between songs when engaging in song alternation. In addition, the coordination pattern adopted depended on the distance between singers, further supporting the occurrence of a meaningful biological phenomenon.

Several hypotheses have been proposed to explain time-specific vocal coordination (reviewed in: Helfer & Osiejuk, 2015; Todt & Naguib, 2000). Only three, however, predict modulation of the coordination pattern related to the distance between counterparts. The ‘interference avoidance hypothesis’ (Ficken et al., 1974) states that song overlapping impairs signal transmission, and thus it is actively avoided. This hypothesis could partially explain the association between alternation and distance, as it does predict a decrease in song overlap at closer distances, but it does not predict the high song overlap we observed at farther distances. The ‘aggressive signalling hypothesis’ (Mennill & Ratcliffe, 2004) also implies a link between distance and level of overlap, but it only predicts less overlap at closer distance. Finally, the ‘submissive signalling hypothesis’ (Searcy & Beecher, 2009) predicts more overlapping when animals are farther apart, but not the alternation at closer distances. The fact that each of the current hypotheses can only partially explain the observed pattern suggests that multiple, context-dependent strategies could be involved in dyadic singing interactions of the lekking long-billed hermit.

The modulation of coordination strategy with distance could arise if overlapping is used as a signal of dominance or aggressive intent that is not only directed towards interacting vocal counterparts but also to third-party bystanders (i.e. prospecting females or intruding males). If so, the association between distance and coordination could arise as the resolution of a trade-off between maximizing signalling aggressiveness in order to attract potential mates or deter intruders (i.e. advertising signals; Dabelsteen, 2005) and decreasing the costs of engaging in aggressive interactions with neighbours. If song overlapping is a proximity risk signal of aggressive intent in which escalation could imply injury costs (Helfer & Osiejuk, 2015), then vocal alternation would help to avoid this cost. However, alternation would occur at the expense of advertising aggressiveness to bystanders. Individuals might take advantage of the lower risk of aggression when singing at far distances by overlapping other singing males and signalling their aggressive intent to females and intruders. This hypothesis predicts

a higher probability of aggression when overlapping does occur at close proximity. It also predicts that male bystanders would avoid overlapping songs and/or that females would prefer them. Interactive playback tests simulating males engaged in overlapping or alternating singing at various distances could test these predictions.

An alternative explanation for the association between distance and singing coordination is that individuals in close proximity experience similar social environments that modulate their behavioural response. However, social and acoustic environments are heterogeneous across leks (some areas are more active, having more males, intruders, interactions and vocalizations; Stiles & Wolf, 1979). Hence, not all dyads singing in close proximity would experience the same environment, but still show a clear coordination pattern. This alternative hypothesis neither explains how different social or acoustic environments experienced by individuals farther away would also trigger a nonrandom pattern of coordination. The most parsimonious explanation remains that they modulate their behaviour based on the distance of their counterparts.

Other factors could potentially modulate gap duration, such as the presence of females or intruders. Indeed, it is reasonable to expect intruders to affect gap duration for one or even both individuals in a simultaneous singing bout. However, these (or any) unmeasured factors could be responsible for the observed patterns only if they closely covary with the factors we took into account. If not, then no statistically significant differences would have been found. Our experience with this system suggests there are no a priori reasons to expect these factors to covary with the distance of birds during interactive singing bouts or with the type of coordination they are engaging. In fact, it is common to observe that lekking males stop singing during interactions with intruders and females and instead engage in ritualized visual displays (Stiles & Wolf, 1979; Araya-Salas et al., n.d.). Nevertheless, further experimental testing (e.g. playback experiments) controlling other unmeasured factors would help to provide additional evidence to support our conclusions.

Although we found evidence for overlapping and alternating, a substantial portion of simultaneous singing bouts did not conform to one of these patterns, but instead were classified as uncoordinated (56% from the complete data set and 54% from known dyads). Many of these were produced by individuals at far distances from each other. A lack of coordination at far distances could occur because individuals were unable to hear each other and were not responding to each other's song, although this does not explain the detection of uncoordinated bouts from individuals in close proximity. Uncoordinated bouts could also occur if vocal coordination is a facultative behaviour that is not employed in all simultaneous singing bouts. However, the higher duration and variation in gaps of simultaneous singing bouts with no clear coordination pattern indicates that vocal behaviour is modified even when no clear coordination pattern is achieved, which suggests that coordination was attempted in at least some of these cases. Alternatively, coordinated singing may constitute a cognitively demanding behaviour that requires attention both to one's own song and to that of another individual (i.e. limited attention effect; Dukas, 2002). In this scenario, imperfect coordination would be expected to occur at least some of the time even when attempted by the participating birds. The idea that song coordination is difficult to achieve is further supported by the fact that, even in bouts with statistically significant coordination patterns, not all song are overlapped or alternated. Finally, other unmeasured contextual factors, such as the presence of intruders or females, could prevent singers from attending to vocal coordination, which could have also contributed to the occurrence of uncoordinated bouts.

We demonstrate the validity of a randomization test for analysing vocal coordination during bouts of vocalizations from multiple individuals. Based on this approach we show that long-billed hermits engage in context-dependent coordinated singing modulated by the distance between singers. The behaviour seems to be partly mediated by the modification of the timing of the signals. The function of a dual coordination strategy is not fully explained by existing hypotheses, indicating that new hypotheses that better account for the complexity of the behaviour are warranted. Overall, our findings indicate that singing coordination is the product of an active behavioural strategy and provide an analytical approach that could be extended to investigate similar patterns in other taxa.

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Supplementary Material

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