Authors: Michael A. Pardo, Emilee A. Sparks, Tejal S. Kuray, Natasha D. Hagemeyer, Eric L. Walters, Walter D. Koenig
Title: Wild acorn woodpeckers recognize associations between individuals in other groups
Journal: Proceedings of the Royal Society B: Biological Sciences

Detailed Methods

Playback Stimuli

We recorded all but two of the calls used for playback stimuli from January-July 2015 using a Sennheiser ME67 shotgun microphone (Wedemark, Germany) with a Rycote softie windscreen (Stroud, Gloucestershire, UK), and a Marantz PMD661 digital recorder (Kawasaki, Kanagawa, Japan) (48 kHz sampling rate, 16 bits of amplitude resolution). The remaining two calls were recorded from February-March 2016 using a Sennheiser ME62 omnidirectional microphone (Wedemark, Germany) with a windscreen and a Roland R26 digital recorder (Hamamatsu, Shizuoka, Japan) (48 kHz sampling rate, 24 bits of amplitude resolution). Calls were only used as playback stimuli when they were recorded from a single known individual with a relatively high signal to noise ratio. We constructed the playback stimuli in Audacity® 2.1.1. The two calls recorded in 2016 at 24 bits of amplitude resolution were down-sampled to 16 bits using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA) prior to processing.

Playback stimuli consisted of a minute of background noise with a fade-in applied to the first ten seconds, followed by two overlapping calls, followed by thirty seconds of background noise, followed by the same two overlapping calls, followed by a final ten seconds of background noise with a fade-out applied to all ten seconds (Supplemental Fig. S1). We included the initial minute of background noise so that there would not be a rapid onset of background noise at the same time as the onset of the first call. The calls were repeated because pilot data indicated that a single playback often failed to elicit a response, and because a previous study with acorn woodpeckers at Hastings used playback stimuli with a similar design [43]. While acorn woodpeckers most commonly produce single *waka* bouts, they sometimes produce two bouts in succession. All but two calls were filtered with a 200 Hz cutoff and a 6 dB roll off. Two calls had unusually loud low-frequency noise, so we used a 12 dB roll off with the same cutoff frequency. As the minimum frequency of *waka* calls is ~400 Hz, this filter is not expected to cause any noticeable distortion to the calls. The final playback stimuli each consisted of a stereo .WAV file (48 kHz sampling rate, 16 bits amplitude resolution) with one channel for each caller. Prior to overlapping the calls, we normalized each call to the same relative amplitude (-3 dB).

Waka calls consist of a variable number of repeated notes, with the first 1-3 and last 1-3 notes of each call typically being shorter and softer than the rest [25]. We overlapped the two calls in a given stimulus such that the first few "full-volume" notes of one call played before the second call began. This is consistent with natural *waka* choruses, in which one bird often begins calling alone and is then joined by one or more additional callers. We overlapped the calls according to the following rule: if the first call has ≤6 full-volume notes, start the second call after the first full-volume note of the first call. If the first call has 7-10 full-volume notes, start the second call after the first two full-volume notes of the first call. If the first call has 11-14 full-volume notes, start the second call after the first three full-volume notes of the first call. We also ensured that at least some of the notes from each caller did not overlap to maximize the chance that the subject would be able to hear and recognize both callers.

Measuring Degree of Synchrony Within Playback Stimuli

As the degree of temporal synchrony between two overlapping callers could potentially contain information about the likelihood that they belong to the same group, we quantified the degree of synchrony of each of our playback stimuli. Call synchrony can be defined in different ways, so we measured four different metrics of synchrony for each playback stimulus: the average timing of the notes of the second caller relative to the notes of the first caller [36], the consistency of the timing between the notes of the two callers, the proportion of the stimulus during which the callers were overlapping (*Proportion of Overlap*) [36], and the *Lag Time* between the start of Caller 1 and the start of Caller 2 [37] (Supplemental Fig. 1).

In order to measure the average relative timing and consistency of timing of the two overlapping callers within a playback stimulus, we first calculated the angular moment for each note of Caller 2 relative to the immediately preceding and following notes of Caller 1. *Angular Moment* was calculated according to the following equation

$$A = \frac{2\pi(X - R_1)}{R_2 - R_1}$$

where X was the start time of a note from Caller 2, R_1 was the start time of the note from Caller 1 that immediately preceded X, and R_2 was the start time of the note from Caller 1 that immediately followed X (Supplemental Fig. S1) [36]. This metric is expressed as an angle in radians, where a value of 0 indicates that X equals R_1, π indicates that X is midway between R_1 and R_2, and 2π indicates that X equals R_2. We measured X, R_1, and R_2 by generating spectrograms of the playback stimuli in Raven Pro 1.5 (Hann window, window size=1226 samples, DFT=2048, overlap=90%), and using a band-limited energy detector to automatically detect the start and end times of each "*wa*" note, the longer of the two note types in a *waka* call (Supplemental Fig. S1). The detector was set to look for signals between 800 and 2000 Hz, with a minimum duration of 0.06 s and a maximum duration of 0.25 s, a minimum separation distance of 0.021 s, and minimum 70% occupancy above a signal-to-noise ratio threshold of 10. The signal-to-noise ratio was calculated using a sliding noise block of 2.001 s with a hop size of 0.489 s. We checked the results of the automatic detector by eye to ensure that each "*wa*" note was correctly detected. We calculated the circular mean of *Angular Moment* within each playback stimulus, which represented the degree to which the notes of the two callers tended to overlap or interleave on average (average timing of Caller 2 relative to Caller 1). We also calculated the standard deviation of the angular moments for each note of Caller 2, which represented the consistency of the timing of Caller 2's notes relative to Caller 1's notes.

We calculated *Proportion of Overlap* as the duration of time during which Caller 1 and Caller 2 overlapped divided by the total duration of the playback stimulus (Supplemental Fig. S1). In many duetting species, a greater degree of overlap between phrases indicates a less synchronization, because the participants sing phrases in turn to give the impression of one continuous song [65]. However, because *waka* choruses usually consist of a single "phrase" uttered by each participant, it seemed more appropriate to consider higher degree of overlap as indicating greater synchrony. *Lag Time* was the difference between the start time of Caller 2 and the start time of Caller 1 (Supplemental Fig. S1), with a smaller *Lag Time* indicating a more synchronous chorus [37].

Measuring Stimulus Duration and Inter-territorial Distance

The recorded calls used as playback stimuli differed in duration and in the distance between the territory of the caller and the territory of the subject who heard the call. As these factors could potentially influence response to the stimuli, we quantified *Stimulus Duration* and *Inter-territorial Distance.* We measured *Stimulus Duration* as the difference between the start of the first "*wa*" note in a playback stimulus and the end of the final "*wa*" note. The start and end times were determined using the same automatic detector used to quantify *Angular Moment*. We calculated *Inter-territorial Distance* by measuring the Euclidean distances between the granary of the subject's territory and the granaries of each of the caller's territories, and taking the arithmetic mean of these two distances (in control stimuli the callers came from the same group so the two distances were identical).

Adding Synchronicity, Stimulus Duration, and Inter-territorial Distance to the Models

We re-ran all of our original models adding *Standard Deviation of Angular Moment, Proportion of Overlap, Lag Time, Stimulus Duration,* and *Inter-territorial Distance* as fixed effects. Before adding these variables to the models, we transformed them by subtracted the midpoint and dividing by ½ the range, so that each variable would be on a scale from -1 to +1. We could not include *Circular Mean of Angular Moment* in the models because of the circular nature of this variable. However, *Circular Mean of Angular Moment* did not differ significantly between test and control stimuli (Watson's two-sample test, U^2=0.095, P>0.10), suggesting that it is unlikely to have been important.

In the model with *Focal Latency to First Positive Flight* as the response variable, *Stimulus Duration* was the only covariate that was significant (P=0.005) (Supplemental Table S2). *Treatment* was still significant in this model, indicating that the effect of *Stimulus Duration* could not account for the differential response to test and control stimuli. *Treatment* was not significant for any of the response variables measured on the group as a whole (just as in the original analysis).

Recording of Playback Trials

We placed a Sennheiser ME62 omnidirectional microphone (Wedemark, Germany) on a tripod between the center tree and the speaker tree, to record any vocalizations produced by the woodpeckers in response to the playback. The microphone was connected to a Roland R26 digital recorder (Hamamatsu, Shizuoka, Japan) in the second observer's blind, recording at a 48 kHz sampling rate with 24 bits of amplitude resolution.

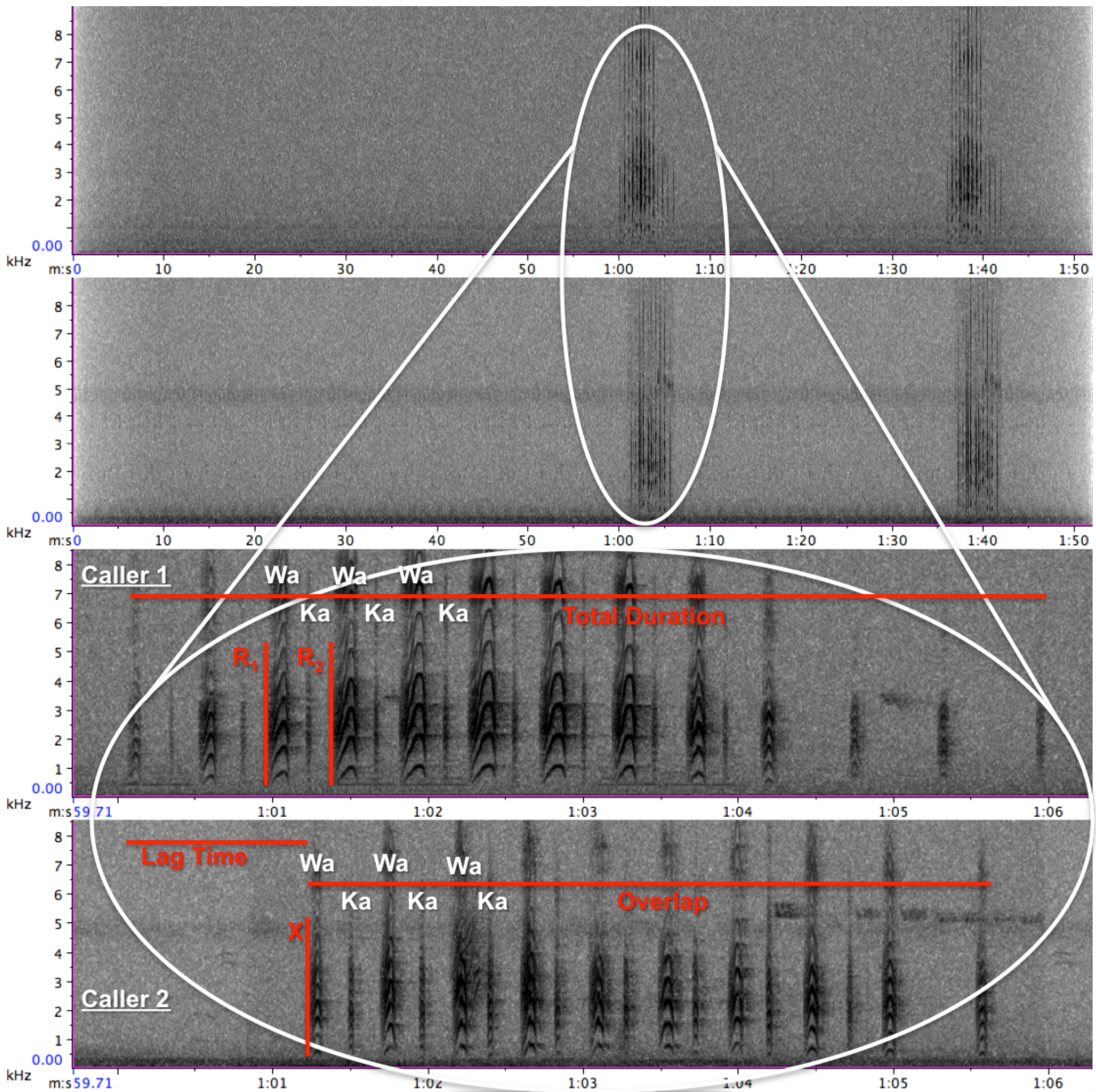
Criteria for Aborting or Redoing Playback Trials

In groups with more than one breeder female, we selected the first breeder female that we saw during the first trial as the focal female for both trials with that group. Before conducting a playback trial, we attempted to ensure that no acorn woodpeckers were in the speaker tree or between the center tree and the speaker tree. If any acorn woodpeckers were spotted in the speaker tree or between the center tree and the speaker during the pre-playback period, then the trial was aborted and not restarted until the birds left the area. We also aborted a trial if a disturbance such as a predator, a loud vehicle, or a territorial intruder appeared during the pre-playback period, or if the focal bird went out of sight during the pre-playback period. If the playback vocalizations had already begun to play by the time we realized that the trial should have been aborted, then the trial was redone after a minimum of two days and the original trial was not used for analysis. We also redid trials if the focal bird did not stay in sight long enough to score most of the response variables of interest.

Characterization of Amplitude of Playback System

In order to determine whether our playbacks were at a naturalistic volume, we measured the amplitude of 26 *waka* calls produced by a captive adult male acorn woodpecker using the SoundMeter 8.4.3 iPhone app (Faber Acoustical, Lehi, UT, USA) with the internal microphone on an iPhone 6. The SoundMeter app was set to flat (Z) weighting, fast response, and Lp level. We held the iPhone 1 m away from the bird with the iPhone's internal microphone pointed towards him and recorded the peak sound pressure level whenever he vocalized. These measurements were not conducted in an anechoic chamber. The amplitude of the *waka* calls that we measured from this individual ranged from 77.7 to 100.1 dB re 20 μPa at 1 m, with a mean value of 92.5 ± 5.4 dB.

After completing the experiment, we also used the SoundMeter app with the same iPhone 6 to characterize the amplitude of the playback system. We placed the speaker in an anechoic chamber 1 m from the iPhone. Both devices were placed on isolation pads on top of pedestals such that the center of the speaker was at the same height as the iPhone, and the speaker and iPhone microphone were facing each other. The SoundMeter app was set to a flat (Z) weighting, fast response, and Lp level, and the speaker was set to maximum gain (as in the original playback trials). Using the same iPod Touch that we used during the original playback trials (set to maximum gain, as in the original trials), we played six of the original playback stimuli three times each and recorded the peak sound pressure level. The mean peak sound pressure level of the playback stimuli was 100.1 ± 1.3 dB re 20μPa at 1 m. Thus, we can be reasonably confident that the amplitude of our playbacks was near the upper end of the range of natural *waka* calls.



Supplemental Figure S1. Spectrogram of a playback stimulus (Hann window, 1135 samples/window, DFT=2048 samples, 80% overlap) with illustrations of how each metric of call synchrony was measured. The top panel displays the full length of the playback file with an initial minute of background noise with a 10 sec fade-in, then the first set of overlapping calls, then thirty seconds of background noise, the same overlapping calls repeated, and 10 final sec of background noise with a fade-out applied to all 10 sec. The bottom panel is zoomed in on the first set of overlapping calls. Each *waka* call consists of a series of alternating “*wa*” and “*ka*” notes, indicated with white labels. The red lines and labels illustrate how each of the metrics of call synchrony (*Angular Moment*, *Proportion of Overlap*, and *Lag Time*) were measured. *Angular Moment* was defined as $2\pi*(X-R_1)/(R_2-R_1)$, and was calculated for each individual “*wa*” note of Caller 2. *Proportion of Overlap* was calculated as overlap/total duration