Supplementary Materials - RSPB-2022-0548

Long-distance vocalizations of spotted hyenas contain individual, but not group, signatures

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1 SUPPLEMENTARY MATERIALS



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Figure S1: Map of the Mara Hyena Project study clans in the Maasai Mara, Kenya. The approximate clan boundaries are indicated by dashed lines. The Talek West (TW) territory lies on the eastern side of the Reserve, approximately 25 km from the remaining three clans which occupy adjacent territories on the western side of the Reserve: Happy Zebra (HZ), Serena South (SS), and Serena North (SN). Reproduced from Green 2015.



Figure S2: Acoustic recordings were obtained from wild spotted hyenas in the field using either (A) handheld shotgun microphone and recording setup deployed from offroad vehicles, or (B) custom-made acoustic and movement recording collars sampling sound directly from the tagged animal.

12 **Recording methods – shotgun microphone**

- 13 Observers deployed recording equipment once the vehicle was turned off and aimed
- 14 the handheld directional microphone (ME-66/K6 and ME-67/K6, sensitivity: 50 mV/Pa,
- 15 frequency response: 40 Hz–20 kHz; Sennheiser Electronic Corporation, Old Lyme)
- 16 toward vocalizing animals within 50 meters of the car. For these recordings, observers
- 17 used a Marantz PMD661 handheld solid-state recorder (Marantz America, Inc., Mahwah,
- 18 NJ; Figure 8a) at sampling rates of 44.1, 48, or 96 kHz and 16 or 24-bit sampling depths.
- 19 From April 2010 to January 2011 recordings were elicited via call-in playbacks (fully
- 20 described in Gersick et al. 2015) and from July 2014 to April 2016 recordings were
- 21 obtained opportunistically. In Table S1, whoop bouts obtained via call-in are italicized
- 22 and those obtained opportunistically are in plain text.

23 Recording methods - collar

- 24 Five collars were deploived from January to March 2017 on five adult females from the
- 25 Talek West clan. Collars consisted of a custom-built DTAG board capable of recording
- audio, accelerometer, and magnetometer data integrated with a GPS board (Gipsy 5;
- 27 Technosmart, Italy) and mounted onto a Tellus Medium collar (Followit Sweden AB).
- 28 Each collar consisted of a base Followit Wildlife (Followit AB, Lindesberg, Sweden)
- 29 Medium Iridium collar with reinforced belting, integrated VHF antenna and a GPS and
- 30 iridium module for transmitting location and battery state. Each collar was fitted with a
- 31 secondary sound and movement module consisting of a modified digital acoustic tag
- 32 (DTAG: Johnson and Tyack, 2003, Johnson et al. 2009) connected using a serial cable
- to a high sample rate Gipsy-5 GPS module (Technosmart Europe, Rome, Italy). This
- 34 module was placed on the top of the collar and thus located on the back of the neck
- 35 with the microphone facing forward and protected by an oleophobic acoustic vent
- 36 (GAW325, 3.2mm ID, W. L. Gore and Associates, Elkton, MD, USA). Collars digitized
- 37 sound using a sigma-delta ADC with an oversampling rate of x6, for a final 32 kHz
- 38 sampling rate and 16-bit depth. In Table S1, whoop bouts obtained via collars are
- 39 bolded.
- 40

41 Table S1. Summary of datasets used in random forest analyses. The clan signature hypothesis was tested

42 with datasets composed of all whoop types (PAST Clan) and only A and S whoop types (AS Clan). The

43 PAST Clan dataset was further divided into females only and males only datasets (see status and sex

44 column). The individual signature hypothesis was tested with datasets composed of all whoop types

45 (PAST Individual) and only A and S whoop types (AS Individual). The PAST Individual dataset was divided

46 into recordings from females only, males only (see status and sex column), microphone only, and collar

47 only. Bolded individuals' recordings were obtained from recording collars.

clan	hyena	status & sex	whoops per bout in dataset				
			PAST clan	AS clan	PAST individual	AS individual	
HZ	JLYR	natal female	8	6			
	PIKE	natal female	6	6			
	SGL-	natal female	5,6	5,6	5,6	5,6	
	SNAP	natal female	8	6			
	ANNR	imm. male	7,10,11	6,7,8	7,10,11	6,7,8	
	ISTA	imm. male	7,6,4,10,13,9	6,5,4,9,10,7	7,6,4,10,13,9	6,5,4,9,10,7	
	JAZZ	imm. male	11,7	10,7	11,7	10,7	
	PGLG	imm. male	5				
	TEMP	imm. male	7	5			
HZ total	9		140	113	106	90	
SN	ANGI	natal female	8	8			
	LOGC	natal female	9	9			
	RBC-	natal female	12	11			
	RMON	natal female	6	6			
	LGO-	imm. male	8,5	7,4	8,5	7,4	
	RALI	imm. male	10	9			
SN total	6		58	54	13	11	
SS	BADG	natal female	7	6			
	BBW-	natal female	6	6			
	BRPH	natal female	7,8,9	6,6,7	7,8,9	6,6,7	
	GRIM	natal female	6	6			

clan	hyena	status & sex	whoops per bout in dataset						
			PAST clan	AS clan	PAST individual	AS individual			
SS	JAVA	natal female	10	9					
	KOMO	natal female	7	7					
	KS	natal female	5	5					
	MTN-	natal female	8	8					
	PALA	natal female	6,3	5,3	6,3	5,3			
	TAJ-	natal female	6	6					
	DEE-	imm. male	6	6					
	ONEK	imm. male	5						
	RSTR	imm. male	11,13,11	10,12,10	11,13,11	10, 12, 10			
SS total	13		134	118	68	59			
TW	BORA	natal female	8,10	7,8	8,10	7,8			
	BYTE	natal female	7,2,2	5					
	FAY-	natal female	2,7,4,1	1,7,1	7,4				
	HRPY	natal female	5	5					
	MGTA	natal female	4,2	3,2					
	ROOS	natal female	12	11					
	TWST	natal female	7,12	6,9	7,12	6,9			
	WRTH	natal female	1,12,1,7,4,1,2	1,12,6,4,1,2	12,7,4	12,6,4			
	DDMA	imm. male	8	7					
	RSWL	imm. male	6,8,2,8,12,9,7,4	5,7,1,6,11,8,5,4	6,8,8,12,9,7,4	5,7,6,11,8,5,4			
	ZITI	natal male	5	5					
TW total	11		182	150	125	98			
Plain text indicate data that were obtained opportunistically from July 2014 to April 2016.									
Italicized lines indicate data obtained from call-ins April 2010 to January 2011.									
Bolded lines indicate data obtained from recording collars January to March 2017.									

48 Acoustic processing methods and feature extraction

- 49 Recordings were resampled to a common sample rate of 32 kHz before being
- 50 processed individually using custom-written software in Matlab 2019a to extract a range
- 51 of acoustic parameters.
- 52 First, each signal was bandpass filtered using a 6-pole Butterworth filter (-3 dB cutoff
- 53 points 100 Hz to 10,000 Hz) and the 99% energy duration of the signal was calculated
- 54 after subtracting average noise energy. Within this time window, a power spectrum was
- calculated using Welch's method (FFT size of 8192, block size 40 ms, block overlap 35
- 56 ms, Hann window), and used to estimate the peak frequency (containing most signal
- 57 energy) and the centroid frequency (separating the power spectrum into two halves with
- 58 equal energy).
- 59 Next, a spectrogram was generated (FFT size of 8192, block size 40 ms, block overlap
- 35 ms, Hann window) and used to extract the fundamental frequency contour using a
- 61 semi-supervised contour tracker. From the contour, 7 features were extracted: The
- 62 minimum and maximum contour frequency were taken directly from the contour. The
- 63 contour was segmented into an initial flat "constant-frequency" (CF) component (ending
- 64 when contour frequency exceeded median frequency by more than 10% of the
- difference between median and maximum frequency) and an upsweep component
- 66 (ending at the point of maximum frequency) and both the total duration and the relative
- 67 time into call where each period ended were extracted, as well as the centroid
- 68 frequency within the initial CF portion (see Figure 1b-d).
- 69 Some whoops contained portions with pronounced subharmonics. To find and quantify
- these events, the acoustic energy in the fundamental frequency, first harmonic and
- second harmonic (1 f0, 2 f0, 3 f0) was isolated and compared to energy in uneven
- harmonics (1.5 f0, 3.5 f0, 3.5 f0) using a variable-frequency filter (Madsen and Jensen
- 73 2012). For the fundamental frequency, -3 dB cutoff points for each time slice
- corresponded to the instantaneous fundamental frequency $\pm \frac{1}{4}$ octave to get a filter
- vidth of 0.5 octave. For higher harmonics, the fundamental frequency was multiplied by
- harmonic number before calculating cutoff points. The total energy was summed across
- even harmonics and across uneven harmonics separately. Then, the ratio of energy
- within harmonics relative to energy within subharmonics was calculated and converted
- to deciBels (10 log10(Eharmonics/Esubharmonics) for both the entire call and for the
- 80 initial constant-frequency component.
- Finally, the signal was downsampled to 8 kHz. For each time slice (40 ms block size, 35
- ms block overlap) within the 99% energy duration of the signal, we calculated the
- 83 spectral entropy (Misra et al. 2004) and cepstral peak prominence (Heman-Ackah et al.
- 84 2014, Soltis et al. 2005) and finally we calculated the mean spectral entropy and
- cepstral peak prominence across the entire signal.

Training Dataset (N-1 individual per clan) Test Dataset (1 individual/clan) Clan 1 ノノヘー Clan 2 Test random forest with the Train with all the whoops from whoops from one individual from n-1 individuals from each clan each clan Results Train 8/11 Test Random Random Forest Forest 2/7

A: Clan identity hypothesis

B: Individual identity hypothesis



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Figure S3: Random forest testing and training dataset for (A) clan signature analysis and (B) individual
signatures analysis. Colored rectangles represent whoop bouts (with variable number of whoops), with
box fill indicating the individual and box border indicating clan. For the clan signature analysis (A), the
random forest was trained using whoops from all but one individual per clan, with the remaining

91 individuals (one per clan) used for testing. The random forest was trained on single whoops and was

92 93 blind to the individual and bout. The random forest was then used to predict the clan membership of each whoop and the proportion of correct guesses was recorded. This procedure avoids pseudo-94 replication of individuals and ensures that the accuracy is not artificially inflated by the model learning 95 signatures of the individuals comprising the clan rather than signatures of the clan itself. For the 96 individual signatures analysis (B), the random forest was trained on whoops from all but one bout per 97 individual, with the remaining bout held out for testing. The trained random forest model was then used 98 to predict the caller of each whoop in the test dataset. This procedure prevents psuedo-replication of 99 bouts and eliminates the possibility of within-bout similarities affecting the analysis of individual 100 signatures.



Figure S4: Confusion matrices for clan signature. (A) Using all whoop types. (B) Using only A and S type whoops. (C) Using all whoop types from females only. (D) Using all whoop types from males only. Rows represent true categories while columns represent the random forest assignments. Each row shows how the random forest classified calls for that particular clan. Each cell represents the percentage of calls that were assigned to the column category from the true category. Guesses that lie along the diagonal are correct assignments while guesses on either side of the diagonal are incorrect assignments. Numbers in white text show the percentage of calls assigned to category x (column x) when it came from category y (row y).





Figure S5: Confusion matrix for individual signature (A) Using all whoop types, (B) Using only A and S type whoops, (C) Using all whoop types from females only, (D) Using all whoop types from males only,

- 114(E) Using all whoop types from microphone recordings, (F) Using all whoop types from collar115recordings. Rows represent true categories while columns represent the random forest assignments.116Each row shows how the random forest classified calls for that particular individual. Each cell117represents the percentage of calls that were assigned to the column category from the true category.118Guesses that lie along the diagonal are correct assignments while guesses on either side of the diagonal119are incorrect assignments. Numbers in white text show the percentage of calls assigned to individual x120(column x) when it came from individual y (row y). Text color indicates clan (black: HZ, blue: SN, green,
- 121 SS, purple: TW), bolded text indicates adult females, and italicized text indicates adult males.

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