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Research Article

Singing in a wolf chorus: structure and complexity of a multicomponent acoustic behaviour

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Abstract

Wolf choruses (*Canis lupus*) are complex, multicomponent signals, composed by a series of different vocalizations emitted by a pack. Although howls, the main component, have been highly studied, poor attention has been drawn upon the other vocalizations of the chorus. In this study, we investigate the structure of the chorus by means of the analysis and the quantification of the different components, taking advantage both of the digital sound recording and analysis, and of the modern statistical methodologies. We provide for the first time a detailed, objective description of the types of call emitted during the wolf howlings, combining spectrographic examinations, spectral analyses and automated classifications, with the aim to identify different types of call. Our results show that wolf choruses have a rich, complex structure, that reveals six other types of call, to be added to those howls already described in literature. Wolf choruses are typically composed by other three different types of calls: the bark, i.e. relatively long calls characterized by low frequencies and the presence of harsh components (deterministic chaos); the whimper, characterized by a harmonic structure and a very short duration; and the growl, a call with a noisy structure, low frequencies but relative long duration. Although further investigations are necessary to understand the meaning of the different calls, this research provides a basis for those studies that aim to compare wolves and other canids vocal behaviour.

Introduction

The wolf (*Canis lupus*) is a gregarious species, whose vocal communication plays a central role in its social behaviour (Harrington and Asa, 2003); wolf vocal repertoire is wide, consisting in 11 and 9 call types emitted by pup and adult wolves respectively (Coscia et al., 1991); these vocalizations have been divided, in connection with the emission circumstances, into short and long range vocalizations (Harrington and Mech, 1978) and into harmonic and noisy sounds (Harrington and Asa, 2003), thus following Morton's motivational-structural rules (Morton, 1977). This graded classification ranges from a friendly/submissive vocalization to an agonistic/aggressive one (Schassburger, 1987, 1993), featuring whine and growl, respectively at the highest and lowest extremities.

Other calls emitted in submissive and friendly contexts are listed as whimper and yelp, while snarl, woof and bark are used in aggressive contexts. However, these classifications are based on a subjective, visual and/or acoustic evaluation and no objective automatic techniques have been applied to determine the different types of call.

Long-distance vocal interactions between timber wolf packs are mostly mediated by chorus howling (Harrington, 1989), a series of vocalizations emitted by a pack, in which one wolf begins howling, followed by some or all the other members, thus forming the chorus (Joslin, 1967). Chorus howlings are complex, multicomponent signals that include several elements (Theberge and Falls, 1967; Harrington and Mech, 1982; Harrington, 1989); indeed, choruses begin with simply-structured howls (Harrington, 1989), but also other kind of calls often occurred in the choral responses (Mech, 1966; Joslin, 1967; Harrington and Mech, 1978; McCarley, 1978) as the chorus progressed.

Within a wolf pack, chorus may be useful to promote the joining of members (Mech, 1966; Theberge and Falls, 1967) and to communicate information on the individual identity and the location (Theberge and Falls, 1967; Tooze et al., 1990; Zaccaroni et al., 2012). Among different packs, chorus serves to mark the territory ownership and occupation, thus minimizing contacts between one pack and another (Joslin, 1967; Harrington and Mech, 1979; Harrington and Asa, 2003).

Howls, the main long range vocalization in wolves have been described in two types: flat, i.e., scarcely modulated, and breaking, i.e., highly modulated and often discontinuous (Harrington and Mech, 1978, 1982; Palacios et al., 2007; Passilongo et al., 2010); more in general, a recent study (Kershenbaum et al., 2016) has found 21 distinct howl types across canid species, confirming and highlighting the complexity and the importance of this call.

Several papers are focused on the acoustic structure of the wolf howls (Theberge and Falls, 1967; Passilongo et al., 2010), highlighting their role as individual' s and pack's vocal signature (Harrington, 1989; Tooze et al., 1990; Passilongo et al., 2012; Zaccaroni et al., 2012) or focusing on the evolution of acoustic repertoire in neonatal period (Coscia et al., 1991; Harrington and Asa, 2003).

While a quantitative and objective method for grouping howls into distinct howl types has been done across canid species (Kershenbaum et al., 2016), no systematic studies have been conducted on the structure and complexity of the whole chorus yet, nor to characterize all the call types present in the chorus.

In this study, we investigate the structure of the chorus by analysing and quantifying the different components. We combine spectro-

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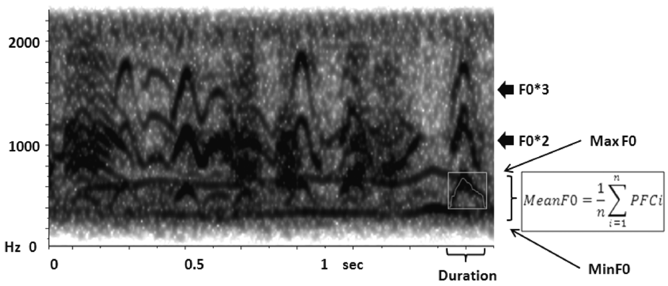

Figure 1 – Spectrogram (DFT size: 2048 samples; Hanning window; frequency grid: 21.5 Hz; time step: 10 ms) showing variables extraction. Selection of the call from the chorus and variables extraction. The average of PFC values corresponds to the mean F0 of the call. Legend: F0=Fundamental frequency; PFC=Peak frequency contour; F0*2=first harmonic; F0*3=second harmonic.

graphic examinations and unsupervised, automated classification techniques in order to examine systematically the qualitative and quantitative acoustic variations in the chorus of free-ranging wolves, with the aim of identifying the different types of call that compose the chorus.

Materials and methods

Study Site and Population

The study area was the province of Arezzo (3230 km^2) in Eastern Tuscany, Italy. Altitude ranges between 300 and 1654 m a.s.l.. Forests are dominated by deciduous trees and cover about 54% of the area. Along this portion of the Apennines, wolves have progressively declined throughout the first half of the last century (Boitani, 1992). In the years of the lowest recorded levels of the Italian wolf population (1950–1970), only a few individuals were reported in these areas (Cagnolaro et al., 1974) and only since the early 1990s the wolf population has been recovering (Mattioli et al., 1995; Apollonio et al., 2004; Mattioli et al., 2004), as a direct consequence of specific conservation laws (Zimen and Boitani, 1975), and also because lands have been abandoned, thus creating better conditions for their survival (Apollonio et al., 2004). The spatial distribution and reproductive success of wolf packs were monitored from 1998 by means of wolf howling, snow tracking, and molecular analysis in the whole province of Arezzo (Gazzola et al., 2002; Apollonio et al., 2004; Scandura, 2005; Capitani et al., 2006; Scandura et al., 2006; Bassi et al., 2015). During the study period on the field (2007–2014), the number of wolf packs ranged from 7 to 11, while the pack size ranged from 2 to 8 individuals.

Data collection

Free-ranging wolves' replies were collected from 2008 to 2014 during a wolf howling monitoring program (following the Habitat Directive on priority species 92/43/ EEC) carried out in the Province of Arezzo. The wolf howling survey consists in the acoustic stimulation produced through human simulation or playback of actual wolf howls (Harrington and Mech, 1979, 1982; Harrington, 1987). This process stimulates the resident wolves to respond to extraneous vocal stimuli in order to defend the resources in their territories and to avoid encounters with neighbour packs. Wolf howling survey was performed in summer (from July to October), when the pack activity was focused in the home-sites, because of the pups presence; so, the rate of response was consequently higher (Harrington and Mech, 1979, 1982; Gazzola et al., 2002). During a howling survey, there is generally no visual access to the replying pack. For this reason, the distance between the operators and the pack has been estimated in two classes: <100 meters (very near chorus, i.e. noise of wolves movements) and between 100 and 500 meters. Details concerning methodology are presented in Passilongo et al. (2010) and Passilongo et al. (2015).

Choruses were captured with a Sennheiser directional microphone fitted with a windshield (ME67 head with K6 power module — frequency response: 50–20000 Hz) and saved on a hand-held M-Audio Microtrack 24/96 II digital recorder, in uncompressed Wave format with a 44100 Hz sampling rate and 16 bits amplitude resolution.

Sound files were initially inspected for quality (i.e. evaluating visually the clarity of the calls for variables extraction). Then, we have selected and analysed 187 calls belonging to 22 choruses emitted by 9 wolf packs.

Sound Analysis

All analyses were performed on a HP Compaq nx7400 laptop computer, using Raven Pro 1.5 (Cornell Laboratory of Ornithology) by means of Discrete Fourier Transformation, the discrete-time counterpart to the continuous-time Fourier series (Charif et al., 2010). DFT size represents the length of the analysis window (the window size), and thus the number of frames sampled to compute each spectrum of the spectrogram (Charif et al., 2008). To analyse calls, parameters were set as follows: DFT size: 2048 samples; Hanning window; frequency grid: 21.5 Hz; time step: 10 ms, where frequency grid = (sampling frequency)/DFT size, while time step was taken to be the distance between the centre of subsequent samples. Calls were selected by visual inspection of the spectrogram. In order to ensure the precision in call detection the harmonic overtones values (integer multiples of the fundamental frequency (F0*2; F0*3; …; F0*N) were checked too. Moreover, all recordings have been scanned by one author (M.M.) and verified by a second one (D.P.).

Source-related parameters

Mean fundamental frequency (MeanF0) values for each call were extracted selecting the call and using "Peak frequency contour"; this command makes a frequency measurement in each spectrogram frame within the selection and reports a vector of frequencies spanning the entire selection (Charif et al., 2010); the average of these values corresponds to the mean F0 of the call. Fig. 1.

Variables included in the analysis were: mean peak frequency contour (MeanF0); standard deviation of frequency contour (SD_F0) minimum (MinF0); maximum (MaxF0) and range (RangeF0) of the fundamental frequency. Duration of the calls (from the beginning to the end of the call selection) was also calculated. The presence of Deterministic Chaos (DC), characterized by widespread energy and weak harmonic structure (Wilden et al., 1998) was also investigated using visual inspection of the narrowband spectrograms.

Intensity

The average energy intensity (dB) of each call was extracted using the "Energy" command in Raven only for within-chorus comparison, as recordings were not normalized according to distance.

Statistical analysis

In order to classify the vocal repertoire, and to identify relatively homogeneous groups of cases, we have used explorative cluster analysis t. A series of agglomerative hierarchical clustering was performed with the "AGNES" (AGglomerative NESting) function in the library "cluster" of R, changing the number of input variables (from 1 to 6) until the highest silhouette value was reached. Ward's method was used to link groups to each other, and the Euclidean squared distance was chosen as a measure of similarity. Silhouette information (0 bad fit, 1 best fit) was computed as a means of interpreting and validating clusters of data (Rousseeuw, 1987). Silhouette plots for different solutions (from 2 to 20 clusters) were compared and the solution with the highest average Silhouette value was chosen as the best.

We have then quantified the distinctiveness of the call types by means of a principal component analysis (PCA), followed by a discriminant function analysis (DFA). The PCA ("princomp", in the default library "STATS") was used to replace the original variables by a smaller set of uncorrelated variables, which are linear composites of the original ones. All acoustic variables were used. We retained principal components with eigenvalues greater than 1 (Kaiser's criterion) for subsequent analyses. The scores of the retained components were tested for normality (Kolmogorov-Smirnov test). A linear DFA (using the "lda" function in the "MASS" library) was then applied in order to validate the cluster classification of call types. Call types were used as

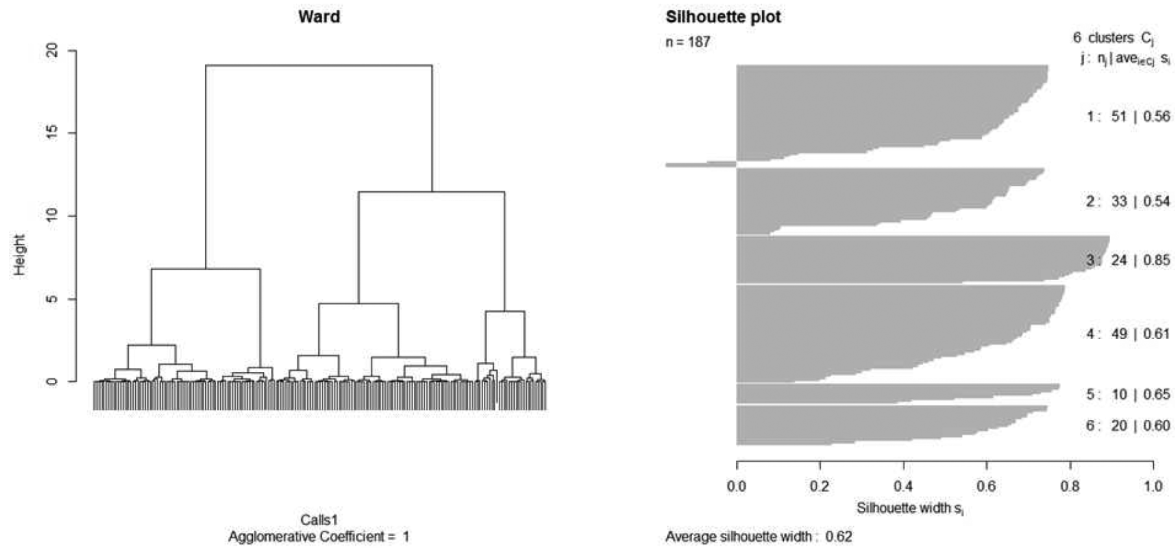

Figure 2 – Cluster tree and silhouette plot. Cluster analysis was used to detect the presence of relatively homogeneous groups of calls. Silhouette Information was computed as a method of cluster interpretation and validation; the highest average silhouette classification score (0.62) was achieved by a six-groups solution based on mean fundamental frequencies as input variable.

the group identifier and the scores of the components were used as discriminant variables.

In order to control the effect of packs and recordings, the acoustic variables of the call types identified in our repertoire classification were compared using linear mixed models (LMM) ("lme" command of "nlme" package for R) with chorus nested within pack as random factors and call type as fixed factor, to the subsequent acoustic dependent variables: Duration, MaxF0, MinF0, RangeF0, MeanF0, SD_F0 and Energy. All analyses were computed in R (version 3.0.1 GUI 1.62 for Windows; The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). All values are reported as mean ± standard error (SE).

Results and Discussion

In the exploratory cluster analysis, the highest average silhouette classification score (0.62) was achieved by a six-group solution (Fig. 2) based on the peak frequency contour (MeanF0). Single silhouette values were 0.56 for the first group (N=51); 0.54 for the second group (N=33), 0.85 for the third group (N=24), while other calls (N=49) were classified in a fourth group (silhouette score=0.61) (Fig. 2). Finally, a small number of calls were classified in the fifth group (N=10, silhouette score=0.65) and in the sixth group (N=20, silhouette score=0.60).

The PCA produced 3 components (PC1–PC3) that exceeded Kaiser's criterion (eigenvalues greater than 1). Together these components explained 86% of the variance. PC1, which explained about 56% of the variance, was mostly related to the MaxF0 and MeanF0. PC2 explained about 17% of the variance and was associated with the MinF0 and SD_F0. Finally, PC3 explained almost 13% of the variance and was related to the Duration. The DFA based on the first three principal components attributed 90.3% of the calls to the group previously identified with the cluster analysis (Fig. 3).

Following the previous nomenclature of wolf's vocal repertoire (Schassburger, 1993), the calls contained in the first group were identified as barks: they are relatively long calls (0.31±0.04 s), characterized by low frequencies (401±11–565±16 Hz) and the presence — in some cases (31%) — of short segments of deterministic chaos, that gives a noisy sound (bark-growl).

Calls contained in the second group were identified as whines, with a harmonic structure characterized by a duration of 0.12±0.09 s and a highly-modulated structure (SD_F0±SE=86±18 Hz). The vocalizations in the third group were identified as growls, since these calls show deterministic chaos and were the lowest in frequencies (211±29–322±42 Hz), and lowest in energy (68.34±5.06 dB) but showed the longest duration (0.42±0.09 sec). The fourth group contained the calls that we have identified as whimpers, with very short duration (0.14±0.08 s) and frequencies that range from 534±26 Hz up to 779±38 Hz and a harmonic structure. Whimper with deterministic chaos are present in 14% of the cases. The calls falling within the fifth group were squeaks. These vocalizations are short (0.19±0.12 s) and show the highest frequencies (1011±36–1353±53 Hz), with no deterministic chaos. Finally, the sixth group is represented by the vocalization called yelps. They are very short calls (0.13±0.10 s) and their high frequencies range from a minimum of 779±30 Hz to a maximum of 1159±44 Hz. Their structure is harmonic with no deterministic chaos.

The statistics associated with the LMM used to compare the acoustic variables among types of call are reported in Tab. 1. There were highly significant differences between call types for each of the analysed variables (Tab. 1 and Fig. 4), including those not included in the explorative cluster analysis.

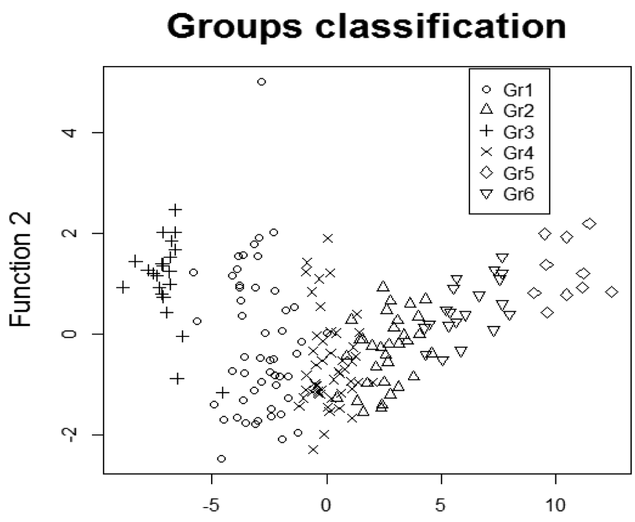

Figure 3 – Two-dimensional scatter plot illustrating the distribution of the call groups against the first two functions of the DFA. 90.3% of the calls have been attributed to the group previously identified with the cluster analysis.

Table 1 – Comparison of the acoustic variables (estimated marginal means ± SE, F and p values) between the six call types identified in the automated cluster analysis and discriminant function analysis.

	Duration (s)	MaxF0 (Hz)	MinF0 (Hz)	RangeF0 (Hz)	MeanF0 (Hz)	SD_F0 (Hz)	Energy (dB)
Bark	0.31±0.04	565±16	401±11	162±21	490±6	32.96±7.60	79.10±3.28
Whine	0.13±0.10	1159±44	779±30	383±54	979±19	98.42±20.00	77.38±5.31
Growl	0.42±0.09	322±42	211±29	108±51	256±18	27.04±19.04	68.34±5.06
Whimper	0.14±0.08	779±38	534±26	242±46	659±16	54.13±17.26	82.19±4.85
Squeak	0.19±0.12	1353±53	1011±36	343±63	1208±22	100.14±23.49	74.58±5.78
Yelp	0.12±0.09	960±40	613±27	352±49	805±17	86.12±18.22	80.43±5.01
F(5,161)	6.75333	257.073	264.932	20.2503	1054.29	11.71134	11.786
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Our results showed that choruses of wolves are rich, complex structures, with several other types of call, different from those howls already described. We have identified six types of calls in the wolves chorus, defined by different combinations of fundamental frequency, duration and by the presence or the absence of nonlinear phenomena (deterministic chaos).

Spectrograms of representative examples of each one of the identified types of call are presented in Fig. 5.

The most common vocalizations in the choruses of wolves, a part from the howls, are barks. Although several subtypes have been previously recognized (Schassburger, 1993; Faragó et al., 2014), our automatic classification only supports a subdivision between harmonic barks (not containing deterministic chaos) and barks-growls containing deterministic chaos. This latter sounds noisy and shows a chaotic structure. It is interesting to notice that, although our classification clearly supports a discrete communication system with boundaries between types of call, this class also shows a graded acoustic structure that could evolve in growls.

According to our finding, barks are used by wolves primarily in threat contexts, such as territorial defence or dominance interactions (Schassburger, 1993; Faragó et al., 2014).

The whines are harmonic, short duration calls produced primarily in stressful situations (Faragó et al., 2014), Whines do also appear to be a form of solicitation or frustration (Robbins, 2000).

Other vocalizations commonly used by wolves during the chorus are the whimpers; whimpers have been described as elongated calls that contain a specific rise in frequency at the onset and fall in frequency at the offset (Schassburger, 1993; Tembrock, 1976; Faragó et al., 2014). Interesting, our findings support the existence of this class and reject the hypothesis of merely arbitrary divisions based on human perception for this call (Harrington, 1996).

Growls are well defined vocalization (Silhouette=0.85), characterized by the presence of deterministic chaos with a poorly defined fundamental frequency. Not surprising, growls are present in wolves chorus; indeed, previous studies (Schassburger, 1993; Faragó et al., 2014) described growls in threatening and defensive contexts, such as expressing dominance, territoriality or protection of resources, exactly the same circumstance as inter-group chorus howling.

Concordant with Morton's motivation structural rule (1977), calls defined a "growl", a threatening and defensive call, shows the lowest fundamental frequency. Growl has also the longest duration, as previously reported, and the lower energy compared with the other calls, showing the same pattern than in African wild dogs (*Licaon pyctus*) (Robbins, 2000).

Another group of calls is squeaks; interestingly, these calls are the highest among the fundamental frequencies found in the choruses. Although the small numbers, they are present in mostly of the packs'

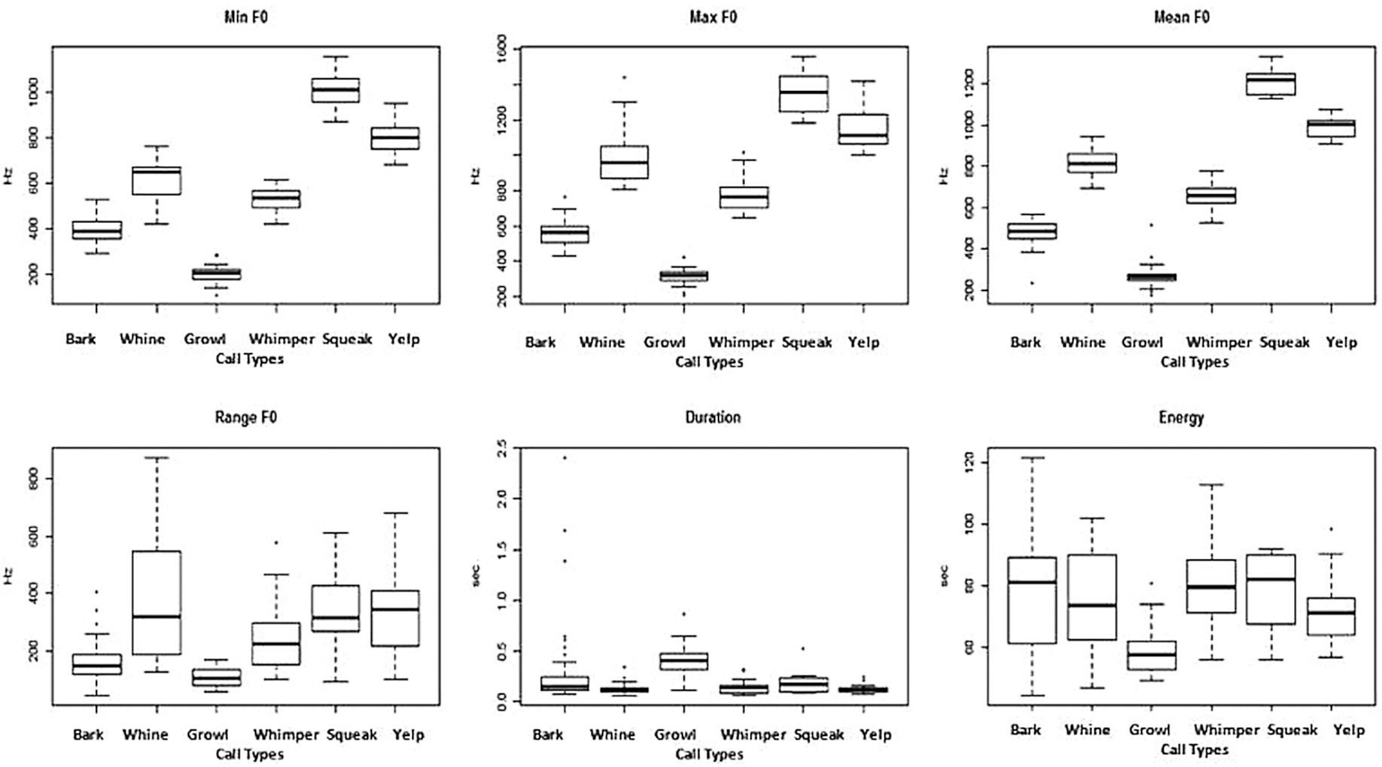

Figure 4 – Box plots illustrating variation of the acoustic variables between call types. Highly significant differences between call types were found for each of the analysed variables.

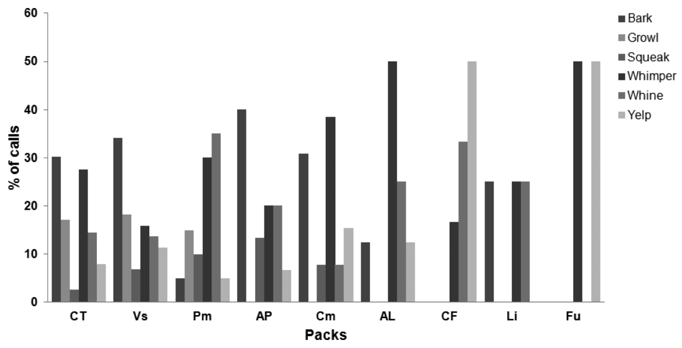

Figure 6 – Histogram representing distribution of the 6 types of call into the 9 wolf packs. At least two call types were found in each pack.

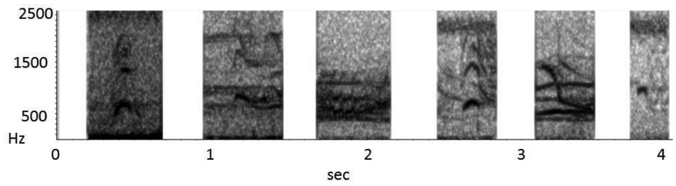

Figure 5 – Spectrograms illustrating the acoustic structure of the six call types: bark (a), whine (b), growl (c), whimper (d), squeak (e) and yelp (f). Calls have been defined by different combinations of fundamental frequency, duration and by the presence or the absence of deterministic chaos.

choruses, confirming that squeaks are prominent features of chorus of wolves.

Finally, we have found yelps, that are characterized by a harmonic structure and a short duration. Although some studies suggested that yelps develop from whines by temporal shortening (Cohen and Fox, 1976; Schassburger, 1993), our automatic classification supports the inclusion of these vocalizations in a different class, because of their frequency and duration.

Whereas some components could be useful in order to transmit information, as the howls (Harrington and Asa, 2003), others could be used in the context of attention-altering signal, (Hebets and Papaj, 2005), as found in coyote barks (Mitchell et al., 2006) or these calls could be related to social interactions between members of the pack, reinforcing relationships and maintaining hierarchies.

Despite different numbers of calls belonging to each pack (13–76 calls/packs), 3 packs (out of the 9 recorded packs) emitted all kind of call; 2 packs emitted 5 types of call, 1 pack emitted 2 types of call, while the two remaining packs emitted three types of call and one very last pack emitted 2 types. They all show a widespread utilization of the different calls (Fig. 5), regardless of the pack.

In our free-ranging-chorus howlings we have not found any vocalization with a maximum fundamental frequency (MaxF0) higher than 1353 Hertz, although wolf vocal extension reach the 9000 hertz (Schassburger, 1993); this discrepancy is probably due to the faster attenuations of the higher frequencies, since the highest frequencies of a signal are subject to a greater attenuation than the lowest (Konishi, 1970; Morton, 1986), and studies on the short range communication are needed for a complete wolf repertoire classifications.

The call types found in the choruses have been previously described as calls used in short range communication. Our results suggest — instead — that they are also commonly present in long range, inter-pack communication.

Our automatic classification could help in adults/pups discrimination, as the acoustic energy distribution is concentrated at higher frequencies when there are pups vocalizing (Palacios et al., 2016). This high frequencies energy can be due to the presence in the chorus of whine and squeak vocalizations, typical vocalizations mostly, but not exclusively (Harrington, 1989), emitted by pups (Coscia et al., 1991).

Contrarily to barks and growls, whine, whimper, squeak and yelp have been described in submissive and friendly contexts (Schassburger, 1987, 1993); playback experiments are now essential to investigate the function of these call types and of their spectral components in both inter than intra packs contest. Many factors, indeed, could affect the type of calls in the choruses (distance between members of the same pack, health and motivational status, etc), so that further investigations are necessary to understand the meaning of the different calls, and to better convey information about wolf vocal behaviours. Comparison with other canid species repertoire with similar acoustic territorial marking behaviour, such as the coyote (*Canis latrans*), and the golden jackal (*Canis aureus*) is clearly needed for a better understanding of canids vocal behaviour.

In conclusion, although further studies involving direct observations are need to understand the function and ontogenesis of these calls, our classification of the structure highlight the complexity and multicomponent nature of the chorus, providing a basis for further researches on wolves and other canids vocal behaviour.

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