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THE ACOUSTIC STRUCTURE OF WOLF HOWLS IN SOME EASTERN TUSCANY (CENTRAL ITALY) FREE RANGING PACKS

D. PASSILONGO[1], A. BUCCIANTI[3], F. DESSI-FULGHERI[*1], A. GAZZOLA[2], M. ZACCARONI[1] AND M. APOLLONIO[2^]

[1] *Department of Evolutionary Biology, University of Firenze, Via Romana 17 I - 50125, Firenze, Italy*
[2] *Department of Zoology and Evolutionary Genetics, University of Sassari, 5 Via Muroni 25, I-07100 Sassari, Italy*
[3] *Department of Earth Sciences University of Firenze Via G. La Pira 4, 50121 Firenze, Italy*

ABSTRACT

Italian wolf howls are described for the first time from observations between 2003–2008 of a population living in eastern Tuscany, central Italy. A sample of 37 howls selected among single responses and 128 howls included in the choruses of 7 free ranging packs was recorded and analysed. The mean fundamental frequency of the howls ranged between 274–908 Hz. Two main structures recognised by means of multivariate explorative analysis, in particular Principal Component and Cluster Analysis, were ascribed to breaking and flat howls. Discriminant Function Analysis was applied to the recognised groups with the aim to find a general rule for classification. Howls with different features were correctly assigned to the groups obtained by explorative analysis in 95.8% of cases. The analysis of the variables characterising the structure of the howls suggests that maximum frequency and range of fundamental frequency are the most important parameters for classification, while duration does not appear to play any significant role.

Keywords: *Canis lupus*, acoustic structure, mammal communication, sonogram, fundamental frequency.

INTRODUCTION

Acoustic signals play an important role in animal communication (Hopp *et al*. 1998). In cooperative as well as competitive contexts, acoustic communication conveys messages rapidly, across long distances and without any physical or visual contact. One of the benefits of acoustic signals is the high degree of variation within each sound type, which enables animals to express variation in meaning (Harrington & Asa 2003). Alarm calls can provide important information about the presence and nature of predators (Melchior 1971) and enable the

receivers to choose the appropriate defence reaction (Sherman 1977; Seyfarth *et al.* 1980; Hoogland 1983). Acoustic communication can also be used to estimate the rivals' size and decide whether or not to fight (Davies & Halliday 1978; Clutton-Brock & Albon 1979; Reby & McComb 2003).

In the wolf *Canis lupus*, a markedly socially gregarious species, the pack is the basic social unit (Mech 1970) and much of their social behaviour is accompanied by vocalizations (Harrington & Asa 2003). Wolf pups vocalize within hours of birth (Coscia *et al.* 1991) and adult wolves' vocal repertoire is wide. Wolf vocal signals have been classified either into short-range and long-range calls (Harrington & Mech 1978a) or into harmonic and noise sounds according to the context, thus ranging from submissive and friendly to aggressive signals (Shassburger 1987; 1993). This last is a graded classification, with whine and growl being listed at its extremes. Other vocalizations are listed as whimper and yelp (in submissive and friendly contexts); snarl, woof and bark (in aggressive contexts); moan (not reported in nature). All of them are used in short-range communication (Shassburger 1993).

The howl is the main long-range vocalization (Harrington & Asa 2003). It is important in both intra and inter-pack communication and has several functions. Within a wolf pack, howling may be useful to promote the joining of members (Mech 1966; Theberge & Falls 1967) and to communicate information on individual identity and location (Theberge & Falls 1967; Tooze *et al.* 1990). Among packs, howling serves to advertise territory ownership and occupation, thus minimizing contact among them (Joslin 1967; Harrington & Mech 1979; Harrington & Asa 2003). Packs are more likely to respond and stand their ground when they are at a fresh kill or accompanied by relatively small pups; in fact, carcasses and pups are resources to be guarded on the spot (Harrington & Mech 1979; Harrington & Asa 2003).

Previous papers have considered the behavioural and ecological issues of wolf howling in relation to the defence of resources and aggressive behaviour (Harrington 1987; Harrington & Mech 1979; 1983), to timing of wolf activity (Harrington & Mech 1978b; Gazzola *et al* 2002; Nowak *et al.* 2007), or to wolf pack census technique (Harrington & Mech 1982; Fuller & Sampson 1988). Some studies considered the acoustic structure of North American wolf howls (Theberge & Falls 1967; Harrington & Mech 1978a; Harrington 1989; Tooze *et al.* 1990); while others, carried out in Eurasia, with the exception of Nikolskii *et al.* (1986) and Nikolskii & Frommolt (1989) that were performed in nature, were limited to captive wolves (Nikolskii & Frommolt 1985; Schassburger 1987; 1993; Frommolt 1999; Palacios *et al.* 2007) and failed to account for the actual influence of captivity on vocalizations (McCarley 1978).

The fundamental frequency (F0) of adults' howls ranges between 150 Hz and more than 1,000 Hz (Theberge & Falls 1967; Harrington & Mech 1978a; Harrington 1989; Tooze *et al.* 1990), which is usually the dominant frequency (Theberge & Falls 1967; Shassburger 1993). The low frequencies and the structure of howls are useful features for long-distance acoustic communication (Harrington & Asa 2003).

The wolf is one of the most widely distributed land mammals. It inhabits all the vegetation types of the Northern hemisphere and environments as different as forests and prairies, tundra, barren ground, mountains, deserts and swamps. Subsequently, the wolf shows high morphological and genetic variability, which accounts for its classification into numerous sub-species (Wayne & Vilà 2003). The Italian Wolf *Canis lupus italicus* is one among six European subspecies, as recently confirmed by means of molecular analysis (Nowak & Federoff 2002). Two of the main physical characteristics of Italian wolves are their lower weight (25–35 kg for adult male) and smaller size (110–148 cm, tail excluded) when compared with North American and Central European populations (Ciucci & Boitani 1998). These two features are also very important parameters as regards the vocalisation process, especially when low frequencies are involved (Morton 1977). Tonal and shape variables, like frequency attributes and modulation, determine – and account for the description of – the structure of howls.

Howls have been described in two forms: flat, i.e., scarcely modulated, and breaking, i.e., highly modulated and often discontinuous (Harrington & Mech 1978a; Harrington & Mech 1982). However, two other forms have been recently described in captive Iberian wolf: continuous wavy and breaking wavy howls (Palacios *et al.* 2007).

This study investigates for the first time the structure of howls in a free ranging Italian wolf population exhibiting inter-pack communication. This is the first study to be conducted on the howls of this subspecies; indeed, it is one of the few studies made on free-ranging European wolves (see also Nikolskii *et al.* 1986; Nikolskii & Frommolt 1989). Therefore, the aims of this paper are: a) to describe the howls of the Italian wolf population and b) to determine how many howl types characterise the Italian wolf.

MATERIALS AND METHODS

Study area

The study area was the province of Arezzo (3,230 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.

Cultivated land and pasture represent 42% of the area and the urban settlement accounts for only 4% of it.

Along this portion of the Apennines, wolves have progressively declined throughout the first half of the last century. 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 has the wolf population recovered (Mattioli *et al.* 1995; Scandura *et al.* 2001; Apollonio *et al.* 2004a; Mattioli *et al.* 2004), as a direct consequence of specific conservation laws.

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 (Scandura *et al.* 2001; Gazzola *et al.* 2002; Apollonio *et al.* 2004b; Scandura 2005; Capitani *et al.* 2006; Scandura *et al.* 2006). During the field study period (2003–2008), the number of wolf packs ranged from 7 to 11, while the pack size ranged from 2 to 8 individuals.

Data collection and sound analysis

To study wolf vocalizations, we followed the "wolf howling" technique, used for the first time by Pimlott (1960) and consisting in the stimulation of resident wolves by tape-recorded playback of wolf howls. This method was employed in several studies involving either wolf pack censuses (Harrington & Mech 1982; Fuller & Sampson 1988) or the acquisition of howling data from captive (Frommolt 1999; Palacios *et al.* 2007) and wild wolves (Harrington & Mech 1978b; 1979; Harrington 1987).

Wolf howling was performed in summer (from June to October), when the pack activity is focused in a restricted area (home-sites) and the rate of response is consequently higher (Harrington & Mech 1978b; 1979; 1983; Gazzola *et al.* 2002; Nowak *et al.* 2007).

Sampling sites were chosen to cover the whole study area: the approach described as "saturation census" by Harrington and Mech (1982) was adapted to local requirements, mainly dictated by the mountainous topography. Sampling sites were chosen to maximize the range of audibility and minimize sound dispersion and their location and number was such as to completely cover the study area. Each session for eliciting howling was a continuous 15 minute-period. If after the first playback stimulus no answer followed, a second trial was attempted 3 minutes later, after which the operators left the site. However, if there was a response we repeated one or more trials from a place closer to the presumed site of response to obtain higher quality recordings.

Concurrent sessions were performed by two groups of operators so as to verify the effective presence of two adjacent packs. We followed the standard procedure suggested by Harrington & Mech (1982). In particular: i) no session was conducted during rain or strong wind; ii) wolf howling was performed overnight, to minimize the noise related to human activities; iii) two trials, the first one lower in volume, were conducted per site. To standardize the stimulus to the wolves, howls were elicited by playback of recorded chorus howls of a captive wolf pair (duration: 1min, 29 sec).

Audio recording were made with a Marantz CP 430 cassette tape recorder from 2003 to 2006. The recordings were digitised using Raven Pro 1.3 (Cornell Laboratory of Ornithology) with 44,100 Hz sampling rate and 16 bits resolution and saved in ".wav" format. From 2007 we used a M-Audio Microtrack 24/96 handheld digital recorder, keeping the same audio file parameters and format as above. All vocalizations were recorded with a Sennheiser ME67 directional microphone with windshield. Only good quality recordings were used for spectrographic visualizations.

Each answer was classified on the basis of the number of vocalising individuals, as either "choral response" (two or more responding individuals) or "single response" (one responding individual). Since the aim of this study was to characterize howls only, the whimpers, barks and growls that often occurred in the choral responses (Mech 1966; Joslin 1967; Harrington & Mech 1978b; McCarley 1978) were excluded from the analysis. From 2003 to 2008 we analysed 37 howls extracted among the single responses by 3 subjects, and 128 howls belonging to the choruses of 7 packs.

We analysed the vocalizations with Raven Pro 1.3 using Discrete Fourier Transformation (2,048 DFT samples, Hanning window, 21.5 Hz frequency grid, 10 ms time step, 37.5 Hz bandwidth). For each howl the fundamental frequency (F0) was sampled every 0.05 seconds with the cursor, using both spectrogram and spectral views (following Tooze *et al.* 1990; Palacios *et al.* 2007). From the analysis of the collected data, 11 variables were obtained and considered useful for a complete investigation of the howls' structure (Table 1). Resolutions and variables were consistent with those used in previous works on wolf vocalizations (Tooze *et al.* 1990; Palacios *et al.* 2007). Amplitude parameters and number of harmonic overtones were not considered since they generally depend on the distance between the animals and the recording site (Harrington & Mech 1978a; McCarley 1978).

Statistical analysis

Normal probability plots were used to explore the variable set, with the aim of characterising the shape of their frequency distribution and

TABLE 1

Variables used in the howl characterisation

Pitch variables	Meanf	Mean of the fundamental frequency calculated every 0.05 seconds (Hz)
	Rangef	Difference between maximum and minimum frequencies (Hz)
	Minf	Minimum frequency of the fundamental one (Hz)
	Maxf	Maximum frequency of the fundamental one (Hz)
	Endf	Frequency at the end of the fundamental one (Hz)
Shape variables	Duration	Duration of the howl (s)
	Posmin	Position at which the minimum frequency occurs (time of Minf/Duration) in the howl
	Posmax	Position at which the maximum frequency occurs (time of Maxf/Duration) in the howl
	Cofv	Coefficient of frequency variation (SD/Meanf) × 100)
	Cofm	Coefficient of frequency modulation $\sum \lvert f(t) - f(t+1) \rvert (n-1)/\text{Meanf} \times 100$
	Abrupt	Number of sudden abrupt changes in frequency (>25 Hz)

correctly choosing central tendency and variability statistics. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis were both used to plot the howl's variance-covariance structures and grouping tendency in a multivariate context.

PCA is a technique able to reduce the original number of variables, forming new uncorrelated variables which are linear composites of the original ones. Only pitch variables and duration were used in order to avoid distortion and to reduce the weight of potential outliers. Hierarchical cluster analysis was carried out to identify relatively homogeneous groups of cases by using the same variables of PCA. Ward's method was used to link groups to each other, while the Euclidean square distance was chosen as a similarity measure. A range of solutions from 2 to 4 clusters was saved in order to investigate the meaning of the multilevel structure of groupings.

Due to the high covariance, plots of the scores of the two principal components represented a fundamental tool to explore the data matrix, particularly when the cases could be discriminated by considering the attribution to the previous cluster solution. In this construction, a cross tabulation procedure clearly showed the nested structure of the clustering process, particularly when different cluster solutions were compared in relation to their relative information. Discriminant Function Analysis (DFA) was used to build a predictive model of group membership based on the 2-cluster solution by using scores of the two principal components as independent variables. Bivariate normality of the groups was checked through Mardia's multivariate test, while equivalence of the covariance matrices was checked through Box's M test. Values of *t* and *Chi* square tests were

computed to explore differences between the two groups, in relation to all pitch and shape variables, and to determine their univariate role in discrimination. Finally, because of the non-homogeneous character of the samples, a Mann-Whitney *U* test was used to compare single and choral howls, pooling data before testing to avoid pseudoreplication (McGregor *et al*. 1992). Statistical analysis was computed with SPSS version 13 (Chicago, Illinois, USA) for Windows and Past version 1.85 (Hammer *et al*. 2001).

RESULTS

Analysed howls show a mean fundamental frequency (FO) value range between 274–908 Hz. F0 of the howls ranges from 21–1,033 Hz, while duration ranges from 0.75–9.55 seconds. The coefficients of frequency variation and modulation spans in the intervals 0.84–45.44 and 0.16–9.09, respectively. Inflexion points of the fundamental (abrupt) range are included in the interval 0–18. The position of the maximum frequency shows a lognormal distribution, so that the maximum F0 occurs during the first quarter in most cases (73% of the howls), while the minimum F0 occurs in the last quarter of the howls in 59% of the sample.

The structure of the howls was determined using PCA and Hierarchical Cluster Analysis as explorative tools. In our sample of 165 howls, PCA indicated that when the two eigenvectors with the highest associated eigenvalues were used, it explained 80% of the total variance.

The first component accounted for about 42% of the variability and was found to correlate with range, maximum of F0 and mean frequency. The second component accounted for about 38% of the variability and was found to correlate with duration (inversely) and minimum and end of F0 (Table 2). A plot of the scores of the two principal components with data discriminated by using a two-cluster solution showed a clear separation which was primarily ascribed to the first component (Figure 1a); on the other hand, plots marked by a three (graph not reported) or four-cluster solution (Figure 1b) displayed separations predominantly due to the second component, as well as a partial overlap between said groups.

Cross tabulation clearly showed the nested structure of the clustering process and, in particular, that cluster 1 tended to include cluster 4, while cluster 2 included cluster 3 completely (Table 3). By taking into account this result and considering that i) most variability was due to the first component and ii) a strict relationship between second component and duration was observed, a two-cluster solution level was preferred to determine the general acoustic structure of recorded howls.

TABLE 2

Values of the variables loads along the two axes generated by Principal Component Analysis with their explained variance. Rangef, Maxf and Meanf are the most important variables to generate the first component (Factor 1) that explain 42% of variability, while Minf, Endf and Duration primarily influenced the second component (Factor 2), explaining 38% of variability. All variables were log-transformed and Varimax rotation procedure applied (for abbreviations see Table 1).

Variables	Factor 1 (42%)	Factor 2 (38%)
Rangef	**0.982**	–0.136
Maxf	**0.933**	0.253
Meanf	**0.738**	0.491
Minf	0.270	**0.740**
Endf	0.232	**0.728**
Duration	0.172	**–0.942**

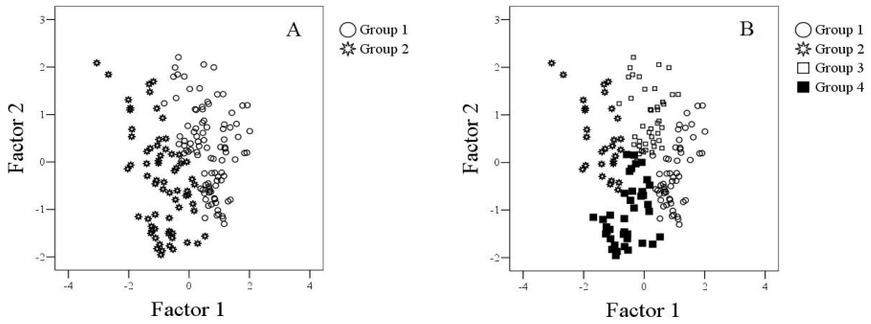

Figure 1. A) Plot of howls grouped by using a 2-clusters solution. Factor 1 and 2 derived by PCA results. Differences between groups of howls are mainly due to the first component -factor 1- (characterized by maximum and range of fundamental frequency). B) Plot of howls grouped by using a 4-clusters solution. Howls in groups 3 and 4 are separated along the second component-factor 2-(characterized primary by duration).

In order to generate a probabilistic rule whereby individual cases could be assigned to the natural groups that had been identified *a priori*, a Discriminant Function Analysis (DFA) was performed. The scores of the first and second components were used as independent variables and the bivariate normality (Table 4) as well as the equivalence of the variance-covariance matrices verified (p = 0.19). The function used to discriminate the *a priori* two groups (Table 5) was able to classify correctly about 95.8% of the howls; about 94.1% of the observations pertaining to cluster 1 were assigned to group 1

TABLE 3

Comparison of data pertaining to the 2 and 4-clusters solution by cross-tabulation, with the emerging of the nested structure. Group 1 and 3 of the 4-cluster solution are fully enclosed in group 1 of 2-cluster solution and groups 2 and 4 of the 4-cluster solution in group 2 of 2-cluster solution.

		– 4 – Cluster solution				Total
	Group	1	2	3	4	
2 –	1	55	0	42	0	97
Cluster solution	2	0	29	0	39	68
Total		55	29	42	39	165

with 5.8% error, while about 96.9% of the observations pertaining to cluster 2 were assigned to group 2 with 3.1% misclassification.

Shape variables that were not used in multivariate analyses to elude possible biases due to numerical constraint (not independent variables) showed higher values in group 1 (from now breaking howls) than 2 (from now flat howls) (Coefficient of variation: t = 11.03, $d.f.$ = 163, $p < 0.001$; Coefficient of modulation: t = 8.09, $d.f.$ = 163, $p < 0.001$). Regarding flat howls, however, our sample shows higher values of Cofv (mean = 9.97%) with respect to the European and North American populations, often having a rise in pitch at the beginning of the howl (Posmax = 0.22) (Figure 2b). The minimum (t = 5.827, $d.f.$ = 163, $p < 0.001$) and the end (t = 5.293, $d.f.$ = 163, $p < 0.001$) frequencies of the fundamental one were higher in the breaking howls, while there were no differences as regards the duration (all variable values split by using DFA solution are reported on Table 6). The same difference between breaking and flat howls was also evident for the number of abrupt changes in pitch (χ^2 = 112.726, $d.f.$ = 14 $p < 0.001$).

DFA correctly classified most howls (95.8%). Flat and breaking howls, however, could not be fully distinguished and represented the two halves of the same continuum (Figure 1a). Duration did not affect

TABLE 4

Mardia's multivariate normality test applied to PCA scores to check bivariate normality assumed by discriminant function analysis; normality has tested on the groups of howls. They are discriminated by considering the 2-cluster solution.

Durnik and Hansen omnibus	Group 1 (N=98)	Group 2 (N=67)
Ep	5.582	3.145
P	0.2326	0.5339

TABLE 5

Eigenvalue of the discriminant function and canonical correlation value. Wilks' lambda and χ^2 statistics are reported.

Function	Eigenvalue	% of Variance	Canonical Correlation	Test of Function	Wilks' Lambda	χ^2	g.l.	p
1	1.032	100	0.835	1	0.303	193.696	2	«0.001

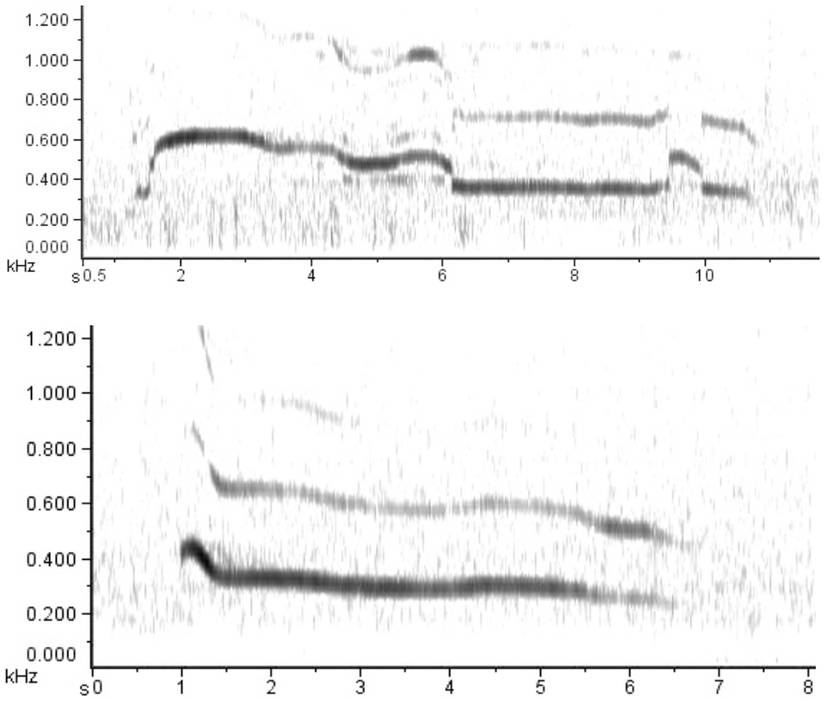

Figure 2. Sonograms representing structures of howls belonging to different groups identified by a 2-cluster solution. Top: example of a breaking howl. Bottom: example of a flat howl. X axis: time in seconds; Y axis: frequency in kiloHertz.

the howls' structures, since breaking and flat howls could both show long as well as short duration (Figure 1b).

Although we could not quantitatively estimate the occurrence of breaking and flat howls in choral responses because we analysed only a selection of good signals, both types of howls were present in all analysed packs. Moreover, breaking howls were more common than flat ones (98 versus 67) and showed higher values in both pitch and shape variables, while flat howls are lower in frequency and relatively constant in form (Figure 2 and Table 6). As regards PCA analysis,

TABLE 6

Grouping according to DFA. Variables list, mean frequencies, standard deviations and range for each group of howls. Shape variables are shown, too. For abbreviations see Table 1.

Variables	Group 1(n=98) Breaking howls Mean ± SD (Range)	Group 2 (n=67) Flat howls Mean ± SD (Range)
Meanf	551 ± 115 (380 -908)	379 ± 72 (274 – 560)
Rangef	428 ± 178 (150 ± 1033)	146 ± 70 (21 – 323)
Minf	366 ± 74 (236 ± 646)	304 ± 74 (193 – 516)
Maxf	794 ± 177 (495 – 1356)	450 ± 80 (301 – 624)
Endf	408 ± 112 (258 – 796)	329 ± 83 (215 – 516)
Duration	3.47 ± 1.60 (0 – 1.00)	4.15 ± 2.19 (0.75 – 9.55)
Posmin	0.75 ± 0.33 (0 – 1.00)	0.57 ± 0.42 (0 – 1.00)
Posmax	0.19 ± 0.22 (0 – 1.00)	0.22 ± 0.25 (0 – 0.98)
Cofv	23.75 ± 8.60 (7.19 – 45.44)	9.97 ± 5.37 (0.84 – 23.96)
Cofm	3.07 ± 1.35 (1.15 – 9.09)	1.79 ± 0.78 (0.16 – 4.41)
Abrupt	4.94 ± 3.08 (0 – 18)	1.51 ± 2.12 (0 – 10)

range and maximum F0 were the most useful immediate variables to classify the structures of howls. In single responses breaking and flat howls were both present (19 breaking and 18 flat), showing that wolves vocalize in modulated as well as unmodulated ways, no matter the type of response (single/chorus). Moreover, when we compared the howls from single responses with those from choral ones, no significant difference was found for all the 11 analysed variables. In fact, single

TABLE 7

Comparison between single and choral howls (for abbreviations see Table 1).

	Meanf	Rangef	Minf	Maxf	Endf	Duration	Posmin	Posmax	Cofv	Cofm	Abrupt
Mann-Whitney U	7	11	11	11	10	9	5	12	12	5	12
P	0.229	0.644	0.644	0.644	0.518	0.405	0.116	0.782	0.782	0.116	0.782

and choral howls did not differ significantly in all analysed variables (Table 7).

DISCUSSION

The higher frequencies of a signal are subject to greater attenuation than the lower frequencies in a variety of environments (Konishi 1970; Morton 1980). Accordingly, it is not surprising that wolf long-distance communication mainly employs low frequencies: the wolf's vocal range is between 70 Hz and more than 9,900 Hz (Schassburger 1993), but only the lower frequencies of this range are actually involved in the production of howls. Despite the great variability of howls according to individuals and social contests, howls are uttered within a narrow range of frequencies, thus experiencing minimum levels of attenuation and proving to be apt for long-distance communication (Harrington & Asa 2003). Our results showed that Italian wolves howl with a mean F0 between 274 and 908 Hz (Table 6), values that are consistent with those reported for other wolf populations (Theberge & Falls 1967; Tooze *et al.* 1990, Shassburger 1993; Palacios *et al.* 2007).

We determined two main structures (Figure 2) corresponding to breaking and flat howls, as already distinguished by Harrington and Mech in their study on North American populations (1978a) where they assumed that lower coefficients of variation (Cofv) correspond to flat howls and higher Cofv to breaking howls (< 6% and > 10%, respectively). Iberian wolf howls showed similar values of Cofv (Palacios *et al.* 2007). The higher values of Cofv found in our flat howls sample is probably due to a characteristic rise in pitch at the very beginning of the howl. At present, we cannot evaluate the importance of this difference concerning flat howls; further investigations that take into account wider areas and samples are necessary to understand whether this high frequency starting can be said to be a systematic feature of Italian wolves.

We were not able to support the division into continuous and breaking howls that was found in the Iberian wolf (Palacios *et al.* 2007), probably because many howls presented both breaking point and modulated fractions (Figure 2). Howls structures were so intrinsically variable that further basic groups could not be identified, with the exception of those based on factor 2, being mainly characterized by duration (Figure 1b).

Duration was an important variable characterizing factor 2 of PCA (Table 2). Duration may be an honest indicator of body size, given that lung capacity limits the airflow necessary to vocalize (Fitch & Hauser 2002), but it does not influence the howls' structure.

As regards the meaning of the two structures, Harrington (1989) suggested that highly modulated howls could serve to disguise inter-

pack information about pack size or to obtain a Beau Geste effect, that is to make the receivers overestimate the number of senders (Krebs 1977). We suggested that those highly modulated howls could correspond to breaking howls. Flat howls, lower in pitch and less modulated, can increase the hostility of the signal (Morton 1977). Birds and mammals use relatively low-frequency sounds in hostile-aggressive contexts and higher frequency sounds in friendly ones. In mammals, the relationship between low frequency sounds and aggressiveness is particularly evident (August & Anderson 1987).

Many factors, however, can affect the type of howls (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 acoustic structures in conveying information about wolf behaviour.

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