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Many bioacoustic studies have been able to identify individual mammals from variations in the fundamental frequency (F_0) of their vocalizations. Other characteristics of vocalization which encode individuality, such as amplitude, are less frequently used because of problems with background noise and recording fidelity over distance. In this paper, we investigate whether the inclusion of amplitude variables improves the accuracy of individual howl identification in captive Eastern grey wolves (*Canis lupus lycaon*). We also explore whether the use of a bespoke code to extract the howl features, combined with histogram-derived principal component analysis (PCA) values, can improve current individual wolf howl identification accuracies. From a total of 89 solo howls from six captive individuals, where distances between wolf and observer were short, we achieved 95.5% (+9.0% improvement) individual identification accuracy of captive wolves using discriminant function analysis (DFA) to classify simple scalar variables of F_0 and normalized amplitudes. Moreover, this accuracy was increased by 100% when using histogram-derived PCA values of F_0 and amplitudes of the first harmonic. We suggest that individual identification accuracy can be improved by including amplitude changes for species where F_0 has only been included so far. Using DFA on PCA values of both F_0 and amplitude could optimize vocal identification in a range of mammal bioacoustic studies.

Keywords: acoustic amplitude; *Canis lupus lycaon*; howl; individuality; vocal recognition; wolf

Introduction

Bioacoustic studies are increasingly being used in population ecology as vocalizations have been found to be highly variable both within and between individuals (e.g. Tooze et al. 1990) and so can be used as a method of individual identification. This vocal individuality can be utilized for monitoring populations remotely over time and can thus be applied to conservation studies (for a review, see Terry et al. 2005). A large range of mammals have been found to show individual identity in their vocalizations including Eastern grey wolves (*Canis lupus lycaon*) (Theberge and Falls 1967), giant pandas (*Ailuropoda melanoleuca*) (Agnarsson et al. 2010) and red squirrels (*Tamiasciurus hudsonicus*) (Digweed et al. 2012). Such individuality is shown in variation in both the fundamental frequency (F_0) and duration of calls (Joslin 1967; Frommolt et al. 2003). A third key component of acoustic communication is amplitude variation within calls (Bradbury and Vehrencamp 1998); however, most studies investigating individual recognition have ignored amplitude data, often because of the

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difficulty of *in situ* recordings (Frommolt et al. 2003) as amplitude attenuates (loses signal) over distance, particularly at higher frequencies (Bradbury and Vehrencamp 1998). Nevertheless, some studies have suggested that amplitude may carry as much individual information as fundamental frequency itself (Mcshane et al. 1995; Charrier and Harcourt 2006). Furthermore, the unequal attenuation of amplitudes between vocalizations can be compensated by measuring changes within amplitudes of individual vocalizations (i.e. normalized amplitude) rather than absolute amplitude data. For example, Charrier and Harcourt (2006) implemented normalized amplitude alongside fundamental frequency changes in Australian sea lions (*Neophoca cinerea*) and found a strong link between both amplitude and frequency modulations and their individual identities. These parameters were used to predict strong individual recognition where the inclusion of amplitude data improved the accuracy of individual recognition over fundamental frequency alone (Charrier and Harcourt 2006). Similar findings have been shown in California sea otters (*Enhydra lutris nereis*) (Mcshane et al. 1995) and giant pandas (Charlton et al. 2009). Therefore, amplitudes may also be useful in improving individual identification accuracy in other mammal species.

Another source of error in many bioacoustic studies is the interference of background noise. Sound analysis programmes address this by using cross-correlation functions but not all achieve the removal of sound that is not harmonic, such as waves on a beach (Schrader and Hammerschmidt 1997). Praat (Boersma and Weenink 2005) is one such commonly applied vocal analysis software program and has been used to extract acoustic features, such as frequency and amplitude, for the analysis of individuality in mammal vocalizations [e.g. red lemurs (*Eulemur rubriventer*) (Gamba et al. 2012), spotted hyenas (*Crocuta crocuta*) (Benson-Amram et al. 2011), goats (*Capra hircus*) (Briefer and McElligott 2011) and giant pandas (*A. melanoleuca*) (Charlton et al. 2009)]. However, Praat is not capable of tracking vocalizations precisely unless it is manually adjusted to get a good fit and may further require specially written code to extract all desired features (Briefer et al. 2012).

The examination of extracted acoustic features from vocalizations for individuality is typically ascertained using a discriminant function analysis (DFA) (Tooze et al. 1990; Darden et al. 2003; Zsebok et al. 2012). This identifies a linear combination of independent variables that best discriminate groups, defined by the user (e.g. vocalizations from individual A), from each other. Simple scalar acoustic variables are singly dimensioned values, describing a characteristic of the data, which are user defined. For example, Palacios et al. (2007) identified mean fundamental frequency, maximum frequency of the fundamental, number of harmonics and frequency modulation as the most important discriminant variables in Iberian wolf (*Canis lupus signatus*) howls.

DFA can also be used to cross-validate the accuracy of individual identification using the selected best combination of variables by comparing predicted group membership (e.g. vocalization belongs to individual A) with actual group membership. However, DFA requires the user to supply the 'group' to which any recordings belong, thus clustering together known vocalizations (e.g. where 'group' might refer to the same individual). Therefore, DFA is a 'supervised' classification technique, requiring the user to identify groups prior to the analysis.

When using simple scalar variables, the user chooses and computes specific scalars, using the time course of the extracted parameters, such as the mean and standard deviation, maximum and minimum values and so on (see Table 1). Although this method is robust and straightforward, it inherently carries the risk that 'some' important information is dismissed from the analysis. To remedy this, the analytical procedure for determining individuality can be refined further by using a principal component analysis (PCA) to reduce the original scalar acoustic variable set to a smaller set of uncorrelated variables (principal components). The first principal component contains the largest variance in the data which accounts for as much of the variability in the data as possible. The principal component values, or 'scores', can be plotted 2D or 3D to show a scatter and, where a scatter groups vocalizations from the same individual more closely than vocalizations from different individuals, identity is suggested (Pearson 1901; Tooze et al. 1990). The PCA values can be fed into a DFA to determine how accurately they can be used to identify individuals, for example if the largest differences are indeed between individuals (Tooze et al. 1990). PCA is not supervised by the observer and does not describe the cause of the deviations in the data, it merely finds them (Pearson 1901).

Theberge and Falls (1967) were the first to suggest that Eastern wolves (*C. l. lycaon*) are able to discriminate between the howls of individuals and packs. Fundamental frequency variation has since been used to identify individuals in three subspecies of wolves: Eastern wolves (Tooze et al. 1990), Iberian wolves (Palacios et al. 2007) and Italian wolves (*Canis lupus italicus*) (Passilongo et al. 2012). The accuracy of individual identification using DFA of simple scalar acoustic variables ranged from 75% (Passilongo et al. 2012) to 86.5% (Tooze et al. 1990), with the most accurate results achieved for captive wolves. However, individual vocalization identity has also been found in other canid species and accuracy has been as high as 99% in swift foxes (*Vulpes velox*) (Darden et al. 2003). Nevertheless, no canid vocalizations have been tested for individuality using a combination of both fundamental frequency and amplitude data. Wolves are a good model species for such a study as their howls have evolved to be transmitted over long distances up to 10 km (Joslin 1967) for territory defence and to communicate individual identity to other pack members (Theberge and Falls 1967). With no visual or olfactory clues available over long ranges, wolf howls may have evolved to carry information about the identity of the individual, its pack and even its current state of arousal (Harrington and Asa 2003). One variable that is known to communicate individual identity in wolves is the fundamental frequency at the position of the maximum amplitude of the howl (Tooze et al. 1990). As the accuracy of individual vocalization identity of wolf howls is currently only 86.5% (Tooze et al. 1990), it is likely that wolf identity could be improved by adding amplitudes to the acoustic analysis.

Therefore, the aims of this study were to:

1. Effectively prevent background noise from adding variation to an analysis by producing a bespoke code designed to extract the fundamental frequency features and amplitudes of the first four harmonics from wolf howls, and comparing this with features extracted using the commonly applied software Praat.
2. Improve the accuracy of individual wolf vocalization identities by including the amplitudes of the first four harmonics of the howls, which are those with the lowest frequency and highest amplitudes.
3. Maximize the efficiency of the search for differences between individuals by adding a new statistical method of histogram-derived PCA values to increase the accuracy of individual identification.

Materials and methods

Source of wolf howls

Eighty-nine howls from six captive wolves were captured on 12 recordings made at Wolf Park, Indiana, between 16 and 29 December 1997. These were recorded on a single microphone set-up: a Marantz PMD-221 recorder and Audio Technica 835A microphone using no parabola; master record number JT9701 on Analogue Cassette at an index of

1430 ms. These were digitized via Studer to A/D board via an Akai cassette into.wav format. All recordings were made by the same observer (J. Tilley) standing next to the enclosure at a distance of no more than 20 m from individual wolves (Monty Sloan of Wolf Park, personal communication). All howls were acquired from the Borror Laboratory of Bioacoustics, Ohio State University, with permission from the copyright holder.

Standard analytical procedure using Praat and DFA

An accepted method for identifying individuality from vocalizations [the free-access speech analysis program Praat (Boersma and Weenink 2005)] was used to extract both fundamental frequency and amplitude features from background noise. Praat outputs fundamental frequency data by fitting points to spectrograms (Skaric 2008). The spectrogram time step was set to 46 ms, and harmonics were fitted to the fundamental frequency and exported as text files. The length of section was a compromise between recordings that were too long, which deteriorate the number of points one can extract along a specific howl, and those that were too short, which deteriorate the frequency assessment. Two howls were excluded from analysis because Praat could not isolate the howls from the background noise.

Bespoke Matlab code

We developed two codes in Matlab® (Mathworks Inc. 2005) for (i) semi-automatic extraction of the time course of both the amplitude and frequency of the vocalization and (ii) further assessment of the benefit of exploiting the amplitude data. We used the same 89 wolf howls to compare features extracted by Praat with those extracted by our own bespoke Matlab-derived code.

Within each howl, the modulus Fourier spectrum of a short section (46 ms) was calculated and stacked along time to obtain a 'spectrogram' (Figure 1).

Howl audio files showed sharp peaks at frequencies that were exact multiples of one another (See Figure 1). The best fitting model between the natural peak shape was a Lorentzian function, defined by:

$$p(f) = A\frac{\gamma^2}{(f - F_0)^2 + \gamma^2},$$

where A is the peak's amplitude, F_0 is the peak's frequency and γ is the Lorentzian's half-width at half of its maximum. We found that fitting the value of γ resulted in spurious results (i.e. the fitted function was often mismatched to the experimental peak), whilst forcing its value to 30 Hz gave excellent match to the vast majority of the data. Note that the value of γ required updating if the frequency resolution (set to 1/46 ms in our case) of the spectrogram was to be changed.

The full function fitted to any instantaneous spectrum, $p(f)$, was the sum of four Lorentzian peaks forced to be exact multiple frequencies of each other, resulting in a five-parameter fitting procedure:

$$P(F_0, A_1, A_2, A_3, A_4) = \frac{A_1\gamma^2}{(f - F_0)^2 + \gamma^2} + \frac{A_2\gamma^2}{(f - 2F_0)^2 + \gamma^2} + \frac{A_3\gamma^2}{(f - 3F_0)^2 + \gamma^2} + \frac{A_4\gamma^2}{(f - 4F_0)^2 + \gamma^2}.$$

Each howl was extracted sequentially as the process was semi-automatic with the user required to define only the exact start and end of the howl within the recording, found by means of showing the user the full spectrogram and having the user define these parameters. The feature extraction then started exactly in the middle of these two user-chosen boundaries, as this was where the signal-to-noise ratio was usually at its best. The user was prompted to check that the first fitted spectrum was correct. Next, the software extracted the rest of the feature fully automatically, moving frame by frame until the end of the howl, then going back to the middle and moving frame by frame to the start. By scanning the spectrogram in this way, and by automatically feeding starting values for the five parameters that were fitted in the immediate neighbour time frame of that being examined, it was found that the fitting procedure was rendered faster and remarkably robust.

Some howls exhibited one or more discontinuities in the time course of the frequency, that is a large, abrupt change in frequency from one time frame to the next, which occasionally affected the feature extraction accuracy. This was tackled using the following strategy: for any particular instantaneous spectrum, an estimate of the frequency of the lowest peak was reliably obtained by identifying the maximum of the cross-correlation function between (i) the data and (ii) the five-parameter function, P, in which the four amplitude values were set to those fitted in the immediate neighbour time frame. Based on this estimate, the frequency and amplitudes of the four peaks with the lowest frequencies were successfully fitted until the entire spectrogram was analysed, thereby providing a data-set matrix of dimension $N \times 5$, where N is the length of the howl divided by 46 ms.

Our code excluded background noise and harmonic sounds, such as bird song, by excluding any sound feature that was not a harmonic multiple of the F_0 of the vocalization. This allowed lower quality recordings containing background noise to be used, excluding the noise from the output file.

Close agreement was found between raw and fitted data (Figure 1). The time course of the resulting five extracted parameters is shown individually in Figure 2. The histograms for the same howl are shown in Figure 3.

Defining individuality through simple scalar variables

The simple scalar variables, identified by Tooze et al. (1990) and Palacios et al. (2007) which are necessary to identify individuals from their howls, were calculated for features extracted by both Praat and the bespoke Matlab code (listed with definitions and abbreviations in Table 1). For the bespoke Matlab code, the simple scalar variables necessary to describe the amplitudes of the first four harmonics were also calculated by normalizing them to the maximum amplitude of each harmonic (Table 1).

Using PCA for automatic identification of deviations defining individuality

For automatic identification of deviations, the data were fed, in the form of a 'training database', to a PCA in Matlab, to enable automated identification of the largest, statistically independent deviations found in the howl database. This supplied the information that may be missing from defined simple scalar variable analysis alone.

The main challenge in this newly developed method was supplying PCA with a training database that did not include the phase lag of the howl relative to the recording start and end (as this information was irrelevant), yet retained the rest of the information. The best results were obtained by computing the histogram distributions of the time courses of the parameters. These histograms (Figure 4) were then stacked and fed as a

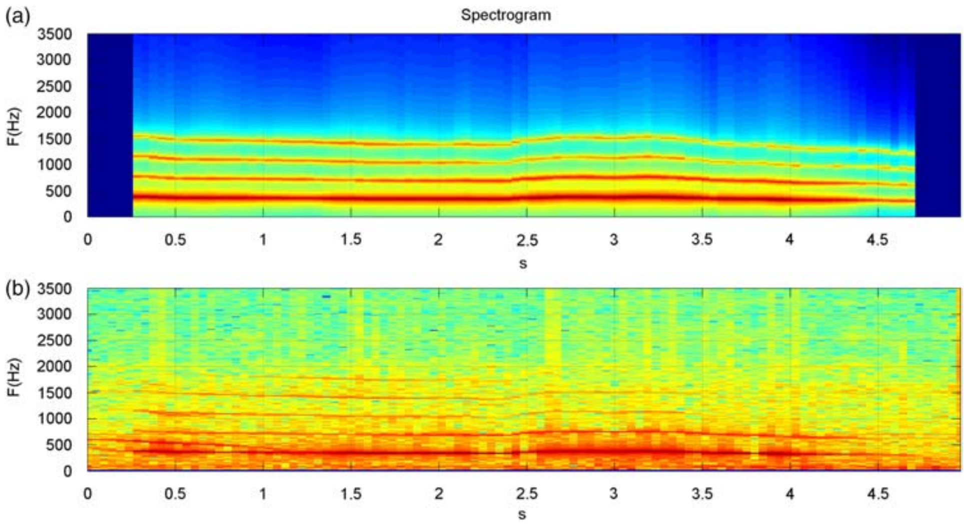

Figure 1. Processed (a) and raw (b) spectrograms from wolf howl 25082:2 extracted using the bespoke Matlab code. The colour codes the sound amplitude on a logarithmic (dB) scale. Note that the other howl present at 0 s is successfully excluded.

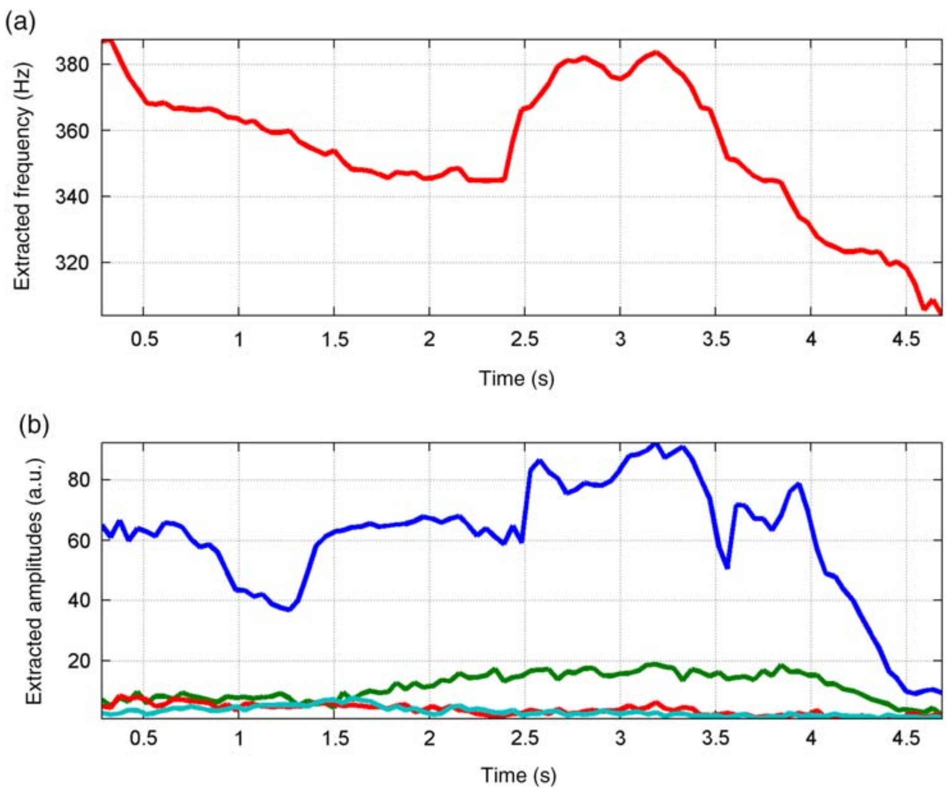

Figure 2. Time course of the fitted frequency (a) and amplitudes (b) for the howl shown in Figure 1: (a) red represents F_0 changes over time; (b) the four colours present the four different amplitudes of harmonics 1–4. Note the independence between the time courses of the four fitted amplitudes on the bottom plot, thereby justifying their individual extraction.

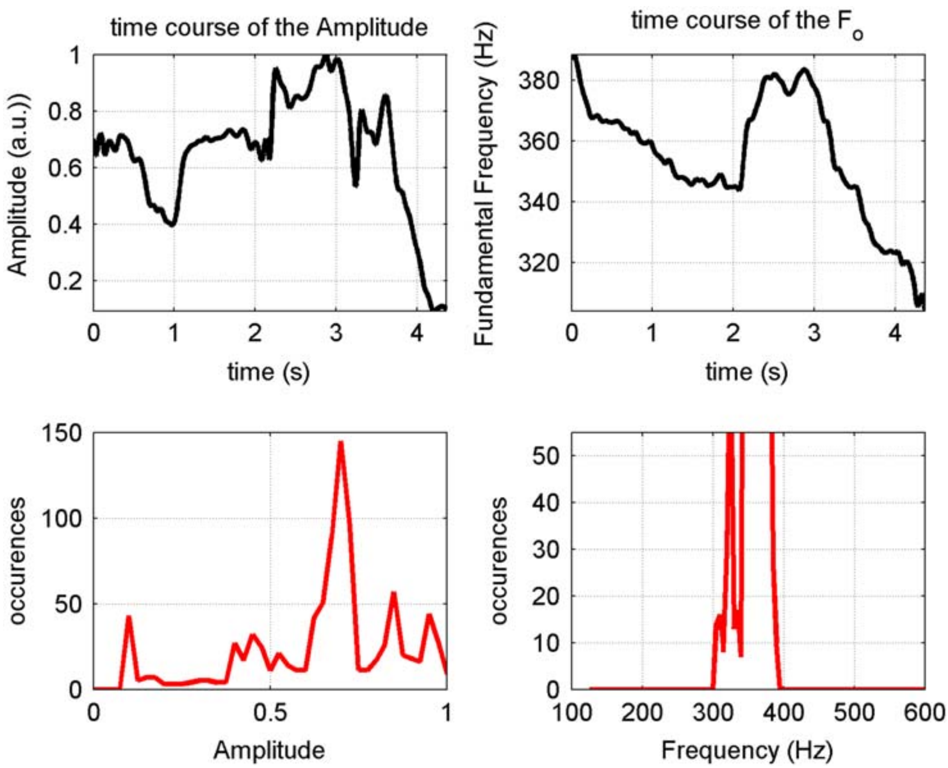

Figure 3. Time course (top graphs) and corresponding probability histograms (bottom graphs) for the amplitude (left graphs) and the frequency (right graphs) of the same howl as shown in Figures 1 and 2. In the histograms, the information regarding the absolute time at which a specific amplitude or frequency occurs is lost, thereby helping the PCA search in identifying relevant deviations.

training database into a PCA search. Smoother histograms were obtained by interpolating the time course data by a factor of 10.

PCA values based on the set of scalars were characterized, and the 40 greatest values were considered for further classification using DFA. Including more PCA values in the analysis added variation to the data-set that did not improve groupings of howls from individual wolves.

PCA values were generated for the six individuals for (i) the 87 howls extracted by Praat, (ii) these 87 howls extracted separately by our bespoke Matlab code and (iii) the full set of 89 howls extracted by our bespoke Matlab code. The PCA values were obtained from the histograms of both the fundamental frequencies (F_0 probability) for all howls and the amplitude of the first harmonic (amplitude probability) for the 89 howls extracted by our bespoke Matlab code. When both F_0 and amplitudes were used together, these were concatenated into arrays of 80 PCA values.

DFA classification of individuals using PCA values and simple scalar variables

For the data-set of 87 howls extracted by both Praat and our bespoke Matlab code, DFA was applied to two sets of descriptive variables: the simple scalar variables and the histogram-derived PCA values describing F_0. For the full set of 89 howls extracted by our bespoke Matlab code only, DFA was applied to three matched levels of analysis: it was

Table 1. Variables of frequency used for individual identification in simple scalar variable analyses.

Variable name	Definition of variable
FreqPaf	F_0 at the position of maximum amplitude of first harmonic
MeanF[a]	Mean frequency of the fundamental at 0.0468 s intervals over duration (Hz)
Maxf[a]	Maximum frequency of the fundamental (Hz)
Minf[a]	Minimum frequency of the fundamental (Hz)
Rangef[a]	Range of the fundamental: range = Maxf−Minf (Hz)
CofM[a]	Coefficient of frequency modulation = $\Sigma\lvert f\,(\mathrm{t}){-}f\,(\mathrm{t}+1)\rvert/(n{-}1) \times 100$ MeanF
CofV[a]	Coefficient of frequency variation = (SD/mean) × 100
Abrupt[a]	Number of discontinuities in the fundamental (change of more than 25 Hz in one time step)
Posmax[a]	Position in the howl at which the maximum frequency occurs: Posmax = time of Maxf/Dur
Posmin[a]	Position in the howl at which the minimum frequency occurs: Posmin = time of Minf/Dur
Endf[a]	Frequency at the end of the fundamental (Hz)
Dur[a]	Duration of the howl measured at the fundamental (s) = t(end)−t(start)
NorAmp1Range	Normalized range of the amplitude of the first harmonic (H1) = range of amplitude of H1/maximum of amplitude of H1
NorAmp2Range	Normalized range of the amplitude of the second harmonic (H2) = range of amplitude of H2/maximum of amplitude of H2
NorAmp3Range	Normalized range of the amplitude of the third harmonic (H3) = range of amplitude of H3/maximum of amplitude of H3
NorAmp4Range	Normalized range of the amplitude of the fourth harmonic (H4) = range of amplitude of H4/maximum of amplitude of H4
NorAmp1Min	Normalized minimum amplitude of the first harmonic (H1) = minimum of amplitude of H1/maximum of amplitude of H1
NorAmp2Min	Normalized minimum amplitude of the second harmonic (H2) = minimum of amplitude of H2/maximum of amplitude of H2
NorAmp3Min	Normalized minimum amplitude of the third harmonic (H3) = minimum of amplitude of H3/maximum of amplitude of H3
NorAmp4Min	Normalized minimum amplitude of the fourth harmonic (H4) = minimum of amplitude of H4/maximum of amplitude of H4
NorAmp2Max	Normalized maximum amplitude of the second harmonic (H2) = maximum of amplitude of H2/maximum of amplitude of H2
NorAmp3Max	Normalized maximum amplitude of the third harmonic (H3) = maximum of amplitude of H3/maximum of amplitude of H3
NorAmp4Max	Normalized maximum amplitude of the fourth harmonic (H4) = maximum of amplitude of H4/maximum of amplitude of H4
NorAmp1Mean	Normalized mean amplitude of the first harmonic (H1) = mean of amplitude of H1/maximum of amplitude of H1
NorAmp2Mean	Normalized mean amplitude of the second harmonic (H2) = mean of amplitude of H2/maximum of amplitude of H2
NorAmp3Mean	Normalized mean amplitude of the third harmonic (H3) = mean of amplitude of H3/maximum of amplitude of H3
NorAmp4Mean	Normalized mean amplitude of the fourth harmonic (H4) = mean of amplitude of H4/maximum of amplitude of H4

[a] Variables used in Praat analysis.

applied to simple scalar variables and histogram-derived PCA values of F_0 alone, amplitudes alone, and F_0 and amplitudes together. The simple scalar variables and PCA values of each howl were labelled with their originator wolf name and the DFA was applied in SPSS (SPSS Inc. 2010).

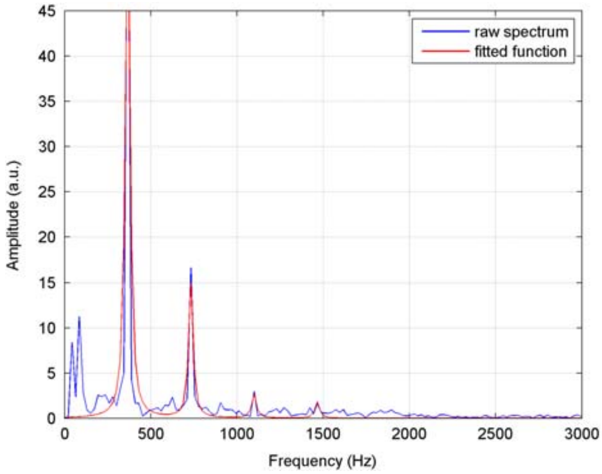

Figure 4. Raw spectrum (blue curve) superimposed with a five-parameter fitted function (red curve) as described in the text, using the same howl as in Figures 1 and 2. Note the remarkable agreement between the fitted curve and the raw data, and the effective dismissal of non-howl-related information, such as the large background noise seen between 0 and 250 Hz.

To optimize the DFA on the simple scalar variables, one-way analyses of variance (ANOVAs) were undertaken in SPSS (SPSS Inc. 2010) on each of the 27 acoustic features to see whether there was a significant difference in acoustic features between individuals which would be useful for DFA (Tooze et al. 1990). Variables that were non-significant were excluded from the DFA.

For the 89 howls extracted via our bespoke Matlab code only, stepwise DFA was then undertaken to establish which variables contributed the most to the clustering and variables were entered in this analysis based on the change in Wilk's λ (F to enter $= 3.84$; F to remove $= 2.71$).

Eight levels of analysis were applied to the data using: 1) the 12 simple scalar variables describing F_0 alone (Table 1), matched with 2) the 40 PCA values describing F_0 alone obtained from the various training databases; 3) the three simple scalar variables describing amplitude change of harmonic one (Table 1), matched with 4) the 40 PCA values describing amplitudes of harmonic one alone; 5) all simple scalar variables of amplitude changes of harmonics one to four; 6) all 27 simple scalar variables describing F_0 and amplitudes of harmonic one together (Table 1), matched with 7) up to 80 PCA values describing F_0 and amplitudes of harmonic one together; and 8) all 27 simple scalar variables describing F_0 and amplitude changes of harmonics one to four.

Results

Choice of significant variables using ANOVA and stepwise DFA

One-way ANOVAs were used to test for differences in the acoustic variables between individuals. For the 87 howls extracted by Praat, 9 out of the 11 variables were significant indicators of individuality and 2 were excluded from DFA: the position in the howl at which the maximum frequency occurs (PosMax) ($F_{5,86} = 0.678$, $p = 0.641$) and the number of discontinuities in the fundamental frequency (Abrupt) ($F_{5,86} = 1.609$, $p = 0.167$). For the matched 87 howls extracted by our bespoke Matlab code, PosMax

($F_{5,86} = 2.217$, $p = 0.060$) and the position in the howl at which the minimum frequency occurs (PosMin) ($F_{5,86} = 1.937$, $p = 0.097$) were also found to be non-significant indicators of individuality so were excluded from DFA. However, Abrupt was not excluded ($F_{5,86} = 4.484$, $p = 0.001$), possibly because the code was better at tracking the howls and created less steep jumps than Praat where the howl changed rapidly. For the full data-set of 89 howls extracted by our bespoke Matlab code, PosMax (df = 88, $F_{5,88} = 2.157$, $p = 0.067$) and PosMin (df = 88, $F_{5,88} = 1.902$, $p = 0.103$) were again excluded from DFA. For the amplitude variables, the range of the normalized amplitude of harmonic 3 (Nor Amp3Range) (df = 88, $F_{5,88} = 2.090$, $p = 0.075$) and the minimum of the normalized amplitude of harmonic 3 (NorAmp3Min) (df = 88, $F_{5,88} = 2.131$, $p = 0.070$) were also excluded from DFA.

Stepwise DFA of our bespoke Matlab code's simple scalar variables showed that the four most important variables were the mean of the fundamental frequency (MeanF) (F to remove = 88.321, Wilk's $\lambda = 0.156$), coefficient of variation of fundamental frequency (CofV) (F to remove = 19.919, Wilk's $\lambda = 0.054$), the normalized mean amplitude of the second harmonic (NorAmp2Mean) (F to remove = 10.141, Wilk's $\lambda = 0.039$) and the normalized maximum amplitude of the third harmonic (NorAmp3Max) (F to remove = 10.051, Wilk's $\lambda = 0.039$).

Benchmarking with Praat

Using Praat, 87 of the 89 howls were successfully analysed using nine simple scalar variables to describe the fundamental frequency (Table 1). Two of the 89 howls were excluded because Praat could not reliably extract them due to background noise interference. DFA of F_0 alone achieved 82.8% accuracy of individual identification (Table 2). However, when the histogram-derived PCA values were used in the analysis instead of the simple scalar variables, the accuracy was improved by 11.5–94.3% (Table 2, Figures 4 and 5).

Table 2. Summary of the DFA accuracies using PCA values and simple scalar variables of fundamental frequency (F_0) changes, amplitude changes and both fundamental frequency and amplitude changes together, and the difference between the PCA value and simple scalar variable analyses for 89 howls extracted by the bespoke Matlab code.

	Variables used	Simple scalar variable accuracy (%)	PCA values accuracy (%)	Difference from simple scalar variable (%)
Praat 87 howls	F_0 changes	82.8	94.3	+11.5
Bespoke code 87 howls	F_0 changes	85.1	96.6	+11.5
Bespoke code 89 howls	F_0 changes (analyses 1 and 2)	83.1	92.1	+9.0
	Amplitude changes of harmonic 1 (analyses 3 and 4)	74.2	85.4	+11.2
	Amplitude changes of harmonics 1–4 (analysis 5)	89.9	–	–
	F_0 and amplitude changes of harmonic 1 (analyses 6 and 7)	88.8	100	+11.2
	F_0 and amplitude changes of harmonics 1–4 (analysis 8)	95.5	–	–
	Four best variables for identity: MeanF, CofV, NorAmp2Mean, NorAmp2Max	89.9	–	–

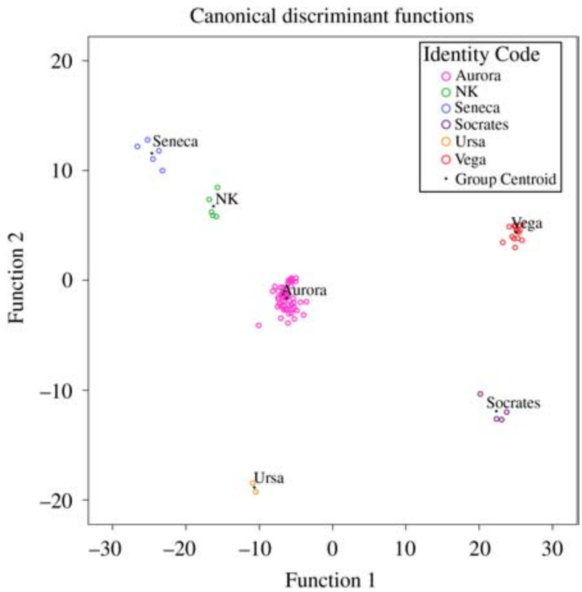

Figure 5. DFA results for correct individual identification from analysis 7 using histogram-derived PCA values of F_0 and amplitude of harmonic 1.

Using the bespoke Matlab code in place of the Praat software improved howl extraction possibilities, allowing extraction of all 89 (100%) howls compared to Praat's 87 (97.8%) (Table 2). When the 87 Praat-extracted howls were matched with the howls extracted with our bespoke Matlab code, individual identification using the significant simple scalar variables of F_0 alone was improved by 2.3–85.1% (Table 2). When the analysis used the histogram-derived PCA values, the accuracy was again improved by 11.5–96.6% (Table 2, Figure 5). This presented a further improvement on the histogram-derived PCA values of Praat-extracted howls by 2.3% (Table 2).

The application of bespoke code to extract howl features

The bespoke code was used to undertake eight analyses on all 89 Matlab-extracted howls (Table 2). The findings show that individual identity was present in the changes of F_0 and amplitudes. Using the four variables found to be most useful by stepwise DFA (MeanF, CofV, NorAmp2Mean, NorAmp3Max), DFA achieved 89.9% accuracy of individual identification using just these simple scalar variables (Table 2). The findings also demonstrate that DFA of histogram-derived PCA values improved on results using the simple scalar variables alone. This suggests that more simple scalar variables are needed to fully describe the howls and to maximize the accuracy achieved.

Discussion

The inclusion of amplitude to improve identification of individual mammals has rarely been attempted due to the difficulty of reliably extracting amplitudes, with distance and background noise confounding fidelity (Frommolt et al. 2003), although this is beginning to change with more studies including amplitude data to improve identification accuracies

(Charlton et al. 2009; Depraetere et al. 2012; Pitcher et al. 2012). Our findings show that including normalized amplitudes improved the individual identification accuracy of wolves in DFA of both simple scalar variables and histogram-derived PCA values. The previous best accuracy for captive Eastern wolves using F_0 alone was 86.5% (Tooze et al. 1990). However, our accuracy of 100% (achieved with histogram-derived PCA values and our bespoke Matlab code) cannot be improved further and is the highest accuracy recorded compared to other canid species where F_0 alone was used (Darden et al. 2003), and to other species where amplitude changes have been used in addition to F_0 (Charrier and Harcourt 2006; Charlton et al. 2009; Rek and Osiejuk 2011).

We have shown that Eastern wolves express individuality in their howls through both temporal changes in F_0 and the amplitude they generate at different points in the howl. However, not all of the amplitude variables are of equal value in identifying individuals, and the amplitude of harmonic 2 appeared to contribute most to identification, shown by stepwise DFA. Consequently, further work could investigate what defines the most important amplitude changes and how these arise, and the effect of distance on the transmission of the amplitudes of the different harmonics. Nevertheless, it is likely that by including amplitudes in analyses of other subspecies of wolves and canids, individual identification accuracy in these species will be improved further. In addition, although our new extraction code is directly applicable to the harmonic vocalizations of canids, the use of amplitudes alongside F_0 to increase the accuracy of individual identification should be extended to other species.

One of the limitations of the approach utilized in this study is that our bespoke code was generated using the licensable software Matlab, whereas the less accurate but more accessible Praat software is free. However, our bespoke code achieved better extraction (100% of howls compared to 97.8%) and produced an automatic fit that also extracted amplitudes. In addition, our bespoke code achieved higher individual identification accuracy for F_0 alone (+2.3%) and achieved 100% accuracy in identifying individuals when it extracted amplitude alongside F_0 data. Again, this suggests that other species would also benefit from code specifically designed to extract their vocalizations. For long-range vocalizations of canids, our code could be used to improve identification accuracy, especially where background noise has previously prevented good-quality extraction of data, for example in barking foxes (*Alopex lagopus*) (Frommolt et al. 2003) where the amplitudes of recordings were affected by the sounds of waves on the beach.

Comparing the DFA findings for simple scalar variables and histogram-derived PCA values, it can be seen that when PCA values of F_0 or amplitudes were used, PCA achieved a higher individual identification accuracy than simple scalar variables, suggesting that further simple scalar variables should be added to describe howls if using this method alone. However, these two systems can be seen as complementary rather than antagonistic as although histogram-derived PCA values show a more complete image of the differences that exist between individuals, they do not provide information on how these differences are defined. Therefore, using histogram-derived PCA values in conjunction with simple scalar variables allows a fuller picture to emerge. We suggest that using histogram-derived PCA values could improve the accuracy of individual identification in mammals by identifying a larger number of significant deviations between individuals that may not be represented by simple scalar variables alone.

Amplitudes are seldom used in bioacoustic studies because of the difficulty in reliably extracting them and controlling the conditions that they are recorded under, and because they attenuate over distance, although this does not mean that the information they carry is always lost (Lameira and Wich 2008). This study adds to an increasing evidence that amplitudes do encode information about individual identity (Charrier and Harcourt 2006; Charlton et al. 2009; Pitcher et al. 2012), although these have rarely been tested at distance. It would be advantageous to have absolute knowledge of the individual wolf howling as they often use howls to communicate over long distances with pack-mates and potential breeding partners (Joslin 1967). However, the application of amplitudes *in situ* requires more work to establish the rate of attenuation over distance and through different habitats, and how far this is affected by individuals, either consciously or through vocal tract differences (Bradbury and Vehrencamp 1998). For example, amplitude measurements may function better in certain environments with few obstacles between subject and observer but should be used with caution for species with high-frequency calls or those in highly heterogeneous environments. The next step is to demonstrate whether including amplitudes could be effective in identifying wild wolves. For these, the distance between observer and wolf would, by necessity, vary substantially and it would be important to show whether the amplitudes would remain reliable indicators of wolf identity. It is expected that they should be as robust to distance as orangutan calls are (Lameira and Wich 2008).

We limited our study to solo howls from individuals so as not to introduce any problems of crossover, seen in chorus howls, affecting amplitudes. Reliably extracting amplitudes from these more complex recordings poses a future challenge. However, Palacios et al. (2012) used chirplet transformation of recordings to separate and extract howls within choruses, where multiple wolves were howling at the same time, and their howls could not easily be separated. We suggest that using histogram-derived PCA values with this, or a similar technique, could allow the reliable separation and classification of howls to individuals using F_0 alone. This method could then be optimized by adding amplitude changes to the analysis.

Charrier and Harcourt (2006) were the first to use normalized amplitude data when using *in situ* wild recordings. Further work could focus on extending our result to wild wolves and identifying differences between vocalizations of different wolf sub-species, packs and possibly genders. We propose that the use of amplitude data in captive mammal populations, where attenuation and degradation will be minimized, will be beneficial to studies trying to identify individuals from vocalizations. However, there have been few studies that have focused on captive and wild recordings of mammal species. Extending these results to other species, in particular canids known to carry individual identity information in their long-distance vocalizations such as coyotes (Mitchell et al. 2006) and African wild dogs (*Lycaon pictus*) (Hartwig 2005), could be possible. By improving the accuracy of individual identity, further insights into species' behavioural ecology may be made, similar to those reported for social learning in birds (Brumm and Slater 2006).

Conclusion

We have demonstrated that our new bespoke Matlab code has substantially improved both the extraction of acoustic features of Eastern wolf howls and the accuracy of individual identity. Furthermore, we believe that using our combination of bespoke code to extract the features and the addition of histogram-derived PCA values could substantially improve individual identification accuracies in other mammal species.

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