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Identifying individual wild Eastern grey wolves (Canis lupus lycaon) using fundamental frequency and amplitude of howls

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The use of amplitudes to identify individuals has historically been ignored by bioacoustic researchers due to problems of attenuation. However, recent studies have shown that amplitudes encode identity in a variety of mammal species. Previously, individuality has been demonstrated in both fundamental frequency (F_0) and amplitude changes of captive Eastern wolf (*Canis lupus lycaon*) howls with 100% accuracy where attenuation of amplitude due to distance was controlled in a captive environment. In this study, we aim to determine whether both fundamental frequency and amplitude data collected from vocalizations of wild wolves recorded over unknown distances, in variable conditions and with different recording equipment, can still encode identity. We used a bespoke code, developed in Matlab, to extract simple scalar variables from 67 high-quality solo howls from 10 wild individuals and 112 chorus howls from another 109 individuals, including lower quality howls with wind or water noise. Principal component analysis (PCA) was carried out on the fundamental frequency and normalized amplitude of harmonic 1, yielding histogram-derived PCA values on which discriminant function analysis was applied. An accuracy of 100% was achieved when assigning solo howls to individuals, and for the chorus howls a best accuracy of 97.4% was achieved. We suggest that individual recognition using our new extraction and analysis methods involving fundamental frequency and amplitudes together can identify wild wolves with high accuracy, and that this method should be applied to surveys of individuals in capture–mark–recapture and presence–absence studies of canid species.

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

Introduction

The science of bioacoustics has developed to enable the vocalizations of different species to be utilized in monitoring populations and in exploring the relationship between the animal and its individual call (Bradbury and Vehrencamp 1998). For example, acoustic sampling has successfully been used to monitor wild populations of bats (O'Farrell and Gannon 1999; Parsons and Jones 2000; Bohn et al. 2007) and marine mammals (Berrow et al. 2009; Frasier et al. 2011). However, application *in situ* is often limited by the accuracy of identification, whether to species, group or individual, so improving this accuracy is vital before surveys that can reliably identify individuals in the wild using vocalizations alone can be undertaken.

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As acoustic monitoring systems become more advanced (Blumstein et al. 2011), recording vocalizations *in situ* has become easier and cheaper, and surveys relying on their analysis is now possible and affordable. The identification of individuals through non-invasive methods such as acoustic monitoring has the potential to produce accurate counts which are vital in conservation studies (e.g. McGregor and Peake 1998) where double-counting and miscounting need to be avoided. For example, a bioacoustic approach has recently been applied to monitor site fidelity in endangered European eagle owls (*Bubo bubo*) (Grava et al. 2008).

Increasingly, researchers have tried to determine whether vocalizations carry information about the individual and whether these can be used as the basis of individual and life history surveys. This has been so successful in bats that entire software programs have been developed around their calls, and a bat can now be identified to species (Parsons and Jones 2000), roost site (Fenton et al. 2004; Jameson and Hare 2009) and kinship group (Boughman 1997) from its echolocation characteristics alone. It is possible that vocalizations of many other species will carry similar information, and therefore bioacoustics has the potential to improve on current animal identification methodologies.

Acoustic monitoring has already been used to explore the distribution of populations of wild canids, often using elicited response techniques to monitor species with large territories (Joslin 1967). Wolves (*Canis lupus*) use howls for three main purposes: territorial defence (Joslin 1967), contacting other members of their own pack (Joslin 1967) and social bonding (Theberge and Falls 1967). With no visual or olfactory clues available over long distances, wolf howls may also have evolved to carry information about the identity of an individual, its pack and even its current state of arousal (Theberge and Falls 1967; Zaccaroni et al. 2012). Indeed, wolves have been shown to display individuality in the variation of both fundamental frequency (Tooze et al. 1990; Palacios et al. 2007) and amplitude (Root-Gutteridge et al. 2013) of their howls, and pack accent has been noted by Passilongo et al. (2010) in wild Italian wolves (*Canis lupus italicus*). Howls from wild wolves have been used to track wolves in presence–absence surveys because they can be heard from distances of 10 km or more (Joslin 1967). These howls have been recorded using both observers with microphones and more recently with automated howl stimulation boxes (Ausband et al. 2011), which are reusable, movable and reliable for elicited wolf howl recordings. However, capture–mark–recapture surveying cannot be undertaken as there is currently no accurate method in place to individually identify wolves based on their howls alone. The accuracy of acoustic sampling of wild wolves is only 75.7% when using fundamental frequency (Passilongo et al. 2012). However, the inclusion of amplitude variables in sound analyses have been shown to be useful in improving identification accuracy in a number of species including California sea otters (*Enhydra lutris nereis*) (Mcshane et al. 1995), giant pandas (*Ailuropoda melanoleuca*) (Charlton et al. 2009) and Australian sea lions (*Neophoca cinerea*) (Pitcher et al. 2012). In addition, when tested over a short distance, amplitudes have been shown to improve the identification accuracy of captive Eastern wolves to 95.5% using simple scalar variables and to 100% when using histogram-derived PCA values (Root-Gutteridge et al. 2013). The accurate identification of individuals in the wild would provide a useful tool in their management and study, and would remove the main criticisms of current simulated howling survey methods (Fuller and Sampson 1988) whereby it is easy to double-count or miscount individuals. Furthermore, as survey techniques may rely on collections of vocalizations made with different equipment, identifying any potential problems of recording fidelity is important.

Wolves howl either alone (solo) or in groups (chorus) where typically all wolves present a howl as part of the chorus (Joslin 1967). Extracting information on individual identity from solo howls poses few problems. However, information from chorus howls is not as easily extracted because, although wolves howling in a chorus howl on different frequencies to form a disharmony (Theberge and Falls 1967), their howls may overlap for brief periods in time and, if there are many wolves howling it becomes difficult to distinguish between them (Tooze et al. 1990). This limits how many howls can be used per chorus, in the absence of identification observations, to one howl per wolf and only as many wolves as can be known to be present because of overlaps in their howls. When using amplitudes, howls that form a chorus are particularly significant as it becomes difficult to know how much each wolf is contributing to the overall amplitude when the howls are both on the same frequency, however, briefly (Theberge and Falls 1967). The analysis of chorus howls is improving with techniques such as Bloodhound, a chirplet-based transformation (Palacios et al. 2012), but they still provide challenges to analyses.

Previously, we developed a bespoke Matlab® (Mathworks Inc. 2005) code for extraction of howls from recordings of captive wolves (Root-Gutteridge et al. 2013), which increased both the number of howls extracted and the accuracy achieved by the free speech analysis program Praat (Boersma and Weenink 2005). For captive Eastern grey wolves (*C. l. lycaon*), discriminant function analysis (DFA) of howls, described using variation in both the fundamental frequency and normalized amplitude, achieved 100% accuracy of individual identification for six wolves (Root-Gutteridge et al. 2013). This presents the most accurate individual identification of a canid species, even improving the 99% accuracy shown in swift foxes (*Vulpes velox*) (Darden et al. 2003). Furthermore, normalized amplitudes were found to increase correct classification using DFA of both simple scalar variables and histogram-derived principal component analysis (PCA) values. However, the recordings were made at a short distance from the howling wolves to minimize interference, and little work has focused on amplitude differences over distance. Whether a similar result could be achieved for wild wolves is unknown as there are problems of amplitude attenuation with increasing distance (Bradbury and Vehrencamp 1998) and interference in amplitude fidelity under both different atmospheric conditions (Bradbury and Vehrencamp 1998) and in different habitats (Charrier et al. 2003).

Therefore, the aims of this paper are as follows:

1. To show whether the bespoke Matlab code developed by Root-Gutteridge et al. (2013) can reliably improve the extraction of sound variables from poor-quality and chorus howls which pose challenges to extraction (Palacios et al. 2012).
2. To demonstrate whether amplitudes can be useful in distinguishing howls of individuals recorded in the wild, and increase the accuracy of identification shown through fundamental frequency alone, with the hope of establishing a baseline for potential *in situ* population surveys.
3. To determine whether differences in microphone type affect individual identification accuracy.
4. To determine whether any differences between wolf pack vocalizations are a result of microphone recording fidelity or pack accent.

Materials and methods

A total of 179 howls from 119 individual wild wolves were obtained from 24 recordings from the British Library Sound Archive, Fred H. Harrington via Public Broadcasting Service (PBS) website and Macaulay Library, New York, with the permission of the copyright owners. The howls were all cited as being from Eastern wolves, and individuals were visually identified at the time of recording. One hundred and fifty-six of the howls were recorded around Algonquin Park, Canada, between May 1959 and 2003. The howls were recorded on six different microphone set-ups in.wav format at 512 bit rate (see Table 1 for details).

Of the 179 howls, we sampled 67 solo howls from 10 individuals, with a minimum of three howls per wolf. These were high-quality individual howls without any background noise and were used to show whether measuring change in amplitude was suitable for identifying individuals in the wild. The remaining 112 howls were taken from a maximum of 109 wolves, with either one or two howls per wolf. These included poor-quality howls, where the recordings were affected by wind or water noise, and chorus howls, where several wolves were howling at the same time, that is where normalized amplitudes were unsuitable for analysis, except for the normalized amplitude of the fundamental frequency. For the chorus howls, only howls that overlapped in time (the second howl starting before the first ended) were used. So, from a potential 40 howls per recording, often only two or three were actually included.

Feature extraction of howls

Howls were extracted from audio files using the bespoke code (Root-Gutteridge et al. 2013) designed in Matlab (Mathworks Inc. 2005) and simple scalar variables were used to describe the features of the fundamental frequency and the amplitudes of the first four harmonics (Root-Gutteridge et al. 2013). Amplitudes of harmonics 2–4 could not be reliably extracted from the chorus howls as, although wolves howl on different fundamental frequencies, they may overlap at points on the same frequencies for the higher harmonics of their howls (Theberge and Falls 1967). Furthermore, poor-quality howls were also expected to have less fidelity in amplitude (Bradbury and Vehrencamp 1998). Therefore, the amplitudes of harmonics 2–4 were only used in the analyses for the solo howls.

Automatic identification of deviations by PCA

The howl feature extraction data were fed, in the form of a training database, to a PCA where the 40 greatest PCA values were considered for further identification using

Table 1. Sources of wolf recordings and number of individuals explored.

Recording area	Recording date	Recording source	Microphone type	Number of howls	Number of individual wolves
Unknown	Unknown	Fred H. Harrington	Unknown model	3	1
Ellesmere Island, Canada	Unknown	British Library Sound Archive	Unknown model	5	1
Algonquin Park, Canada	1980–1995	British Library Sound Archive	Dan Gibson P-650 and Sony P-206, third model unknown (BBC)	80	50 (maximum)
Algonquin Park, Canada	1959–1960	Macaulay Sound Archive	Nagra III recorded by William Gunn	91	67 (maximum)

Note: Wolf identity was established either visually or by only sampling a limited number of howls from a recording.

DFA. PCA values were obtained by two separate strategies: (i) the histograms of the fundamental frequency (F_0 probability) only, undertaken on all 179 howls, and (ii) the histograms of the amplitude of the first harmonic (amplitude probability) only, limited to the 67 high-quality solo howls. Therefore, the 67 solo howls had a total of 80 PCA values (F_0 and amplitude of harmonic one probability) for further identification via DFA.

Classification using DFA

DFA was optimized by using one-way analyses of variance (ANOVA) in SPSS (SPSS Inc. 2010) on all data-sets to determine whether there was a difference within individuals, microphones and packs for each of the 27 extracted simple scalar variables so that only variables that were significantly different within groups were used in the DFA, following Palacios et al. (2007).

Analysis 1: individual identification of wolves from chorus and poor-quality howls

Using bespoke Matlab code (Root-Gutteridge et al. 2013), we extracted acoustic features from 179 howls from a maximum of 119 wolves. DFA was applied to (i) the histogram-derived PCA values and (ii) simple scalar variables describing changes in F_0 only. In addition, DFA was applied to (i) the histogram-derived PCA values and (ii) simple scalar variables describing changes in both F_0 and normalized amplitude of harmonic one (NorAmp1) in an attempt to improve individual identification further (Root-Gutteridge et al. 2013).

Analysis 2: individual identification of wolves from solo howls

A further analysis was made of the 67 solo howls, from 10 wolves, where all amplitudes could be used. Therefore, in addition to simple scalar variables describing F_0 and normalized amplitude of harmonic 1, the normalized amplitudes of harmonics 2–4 (NorAmp2, NorAmp3 and NorAmp4) were included in the DFA. Analyses were undertaken for (i) F_0 alone, (ii) amplitudes of harmonics 1–4 alone and (iii) both F_0 and amplitudes of harmonics 1–4 together. A stepwise DFA was then undertaken to establish which acoustic variables contributed most to the analysis, with variables considered based on the change in Wilk's λ (F to enter = 3.84; F to remove = 2.71).

Analysis 3: identification of wolves using different microphone types

A separate DFA was carried out for each of the three microphones that had recorded howls from more than one individual wolf (see Table 3 for details). Analyses were undertaken using the simple scalar variables describing (i) F_0 alone and (ii) F_0 plus the normalized amplitude of harmonic 1.

Analysis 4: potential microphone and pack differences

DFA was applied to all 179 howls that were recorded using six different microphone types and were from 14 different packs. Analyses were undertaken using the simple scalar variables describing (i) F_0 alone and (ii) F_0 plus the normalized amplitude of harmonic 1.

Results

Following the optimization of extracted sound variables for DFA, only the position in the howl at which the minimum frequency occurs (PosMin) was non-significant for all 119 wolves together ($F_{62,116} = 1.259$, $p = 0.162$), the 10 wolves from the solo howls ($F_{9,66} = 1.806$, $p = 0.087$), the 14 packs ($F_{13,165} = 1.715$, $p = 0.062$) and the six microphone types ($F_{5,173} = 1.724$, $p = 0.131$). This was in agreement with optimization of extracted sound variable from captive Eastern wolves (Root-Gutteridge et al. 2013).

Analysis 1: individual identification of wolves from chorus and poor-quality howls

When all 179 howls from the 119 wolves were analysed together, DFA using F_0 simple scalar variables alone, extracted by the bespoke Matlab code, achieved 82.7% identification accuracy (Table 2). This accuracy was increased to 97.4% when using histogram-derived PCA values, suggesting that individuality is strongly present in howls, despite the quality of howl recording or the extraction of acoustic variables form chorus howls. However, more simple scalar variables are required to define individuality to match the PCA values result.

Analysis 2: individual identification of wolves from solo howls

When the 67 best quality solo howls were analysed with DFA using F_0 simple scalar variables alone, 88.1% identification accuracy was achieved which was further increased to an accuracy of 100% when using histogram-derived PCA values (Table 2). These same percentages were also seen for amplitudes of harmonics 1–4 alone (Table 2). When DFA was applied to both F_0 and amplitudes of harmonics 1–4, identification accuracy was increased to 98.5% (+10.4% over either F_0 or amplitude alone) and further increased to an accuracy of 100% when using histogram-derived PCA values of F_0 and amplitude 1 (Table 2; Figure 1). Therefore, wild wolves, like captive wolves, can be accurately identified from solo howls using changes in both F_0 and amplitude of their howls, indicating that amplitudes carry information on wolf identity.

Stepwise DFA of the bespoke Matlab code's simple scalar variables showed that the four most important variables were the normalized maximum amplitude of the third harmonic (NorAmp3Max) (F to remove = 17.151, Wilk's $\lambda = 0.018$), duration (Dur) (F to remove = 21.847, Wilk's $\lambda = 0.021$), F_0 at the position of maximum amplitude of first harmonic (FreqPAF) (F to remove = 19.311, Wilk's $\lambda = 0.019$) and range of the F_0

Table 2. Summary of the DFAs for individual identification of wild wolves.

Howls used	Variables used	Identification accuracy from DFA using simple scalar variables (%)	Identification accuracy from DFA using histogram-derived PCA values (%)	Difference between DFA using simple scalar variables and histogram-derived PCA values (%)
179 howls, including solo and chorus	F_0	82.7	97.4	+14.7
67 solo howls	F_0	88.1	100	+11.9
	Amplitudes 1–4	88.1	100	+11.9
	F_0 and amplitudes 1–4	98.5	100	+1.5

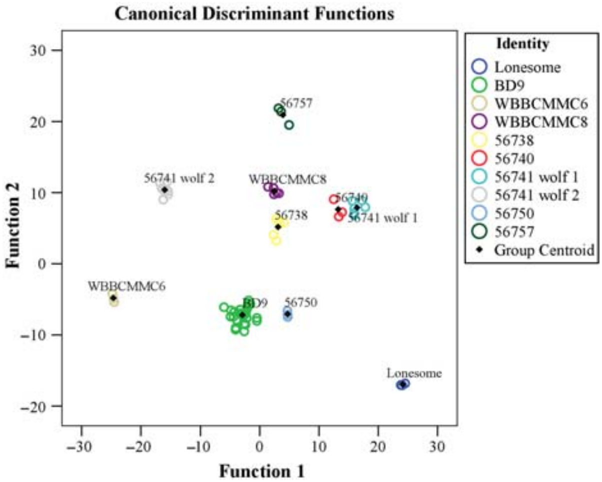

Figure 1. Plot of DFA output using histogram-derived PCA values for 67 solo howls from 10 wolves with 100% accuracy achieved.

(RangeF) (F to remove = 13.764, Wilk's $\lambda = 0.015$). These four variables alone could achieve identification accuracies of 85.1%, compared to 98.5% using all 26 variables.

Analysis 3: identification of wolves using different microphone types

Using simple scalar variables of F_0 alone, the lowest identification accuracy was 82.4%, achieved from the oldest microphone (Nagra III recordings made in 1959–1960), with the newer recordings (on presumably newer microphones, dates not given) achieving 90–100% accuracy (Table 3). However, this could not be separated from the effect of the larger sample size for the Nagra III recordings. When simple scalar variables of normalized amplitude of harmonic 1 were also included in the analyses, accuracies improved (apart from the Dan Gibson P650 microphone which remained at 100%; Table 3). The F_0 alone findings were similar to those for all 179 howls analysed together (82.7%) and for wild wolves (75.7%) (Passilongo et al. 2012). Therefore, it is likely that we detected differences between wolves rather than simply detecting differences in equipment.

Analysis 4: potential microphone and pack differences

Using simple scalar variables of F_0 alone, the different microphones were identified with 74.9% accuracy (Figure 2) and the different packs with 66.5% accuracy (Figure 3).

Table 3. DFA for each microphone type.

	Nagra III	Dan Gibson P650	Unknown BBC model
Number of howls	91	49	10
Number of individuals	67	22	3
Identification accuracy for F_0 only %	82.4	100	90
Identification accuracy for F_0 and HAmp1 %	87.9	100	100

Viewing these figures together, it is clear that the groupings to microphones and packs are too similar to separate the effects of each and to know which is creating the groupings. However, when using simple scalar variables of both F_0 and the normalized amplitude of harmonic 1, the howls recorded on different microphones were identified with 79.9% accuracy (+5.0%) and from different packs with 70.4% accuracy (+3.9%). Pack accent and microphone effect could not be separated further.

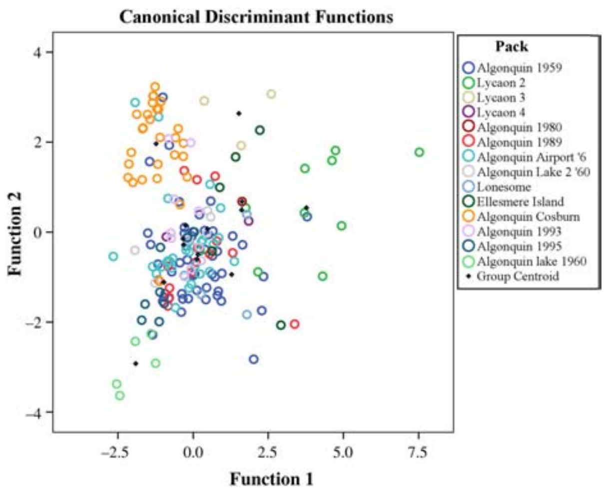

Figure 2. Plot of DFA output for 179 howls recorded across six microphones with 74.9% accuracy in microphone identification, using simple scalar variables. Clustering to microphone is stronger for some microphones (e.g. Nagra III) than others (e.g. Dan Gibson P-650 and Sony P-206 parabola microphones).

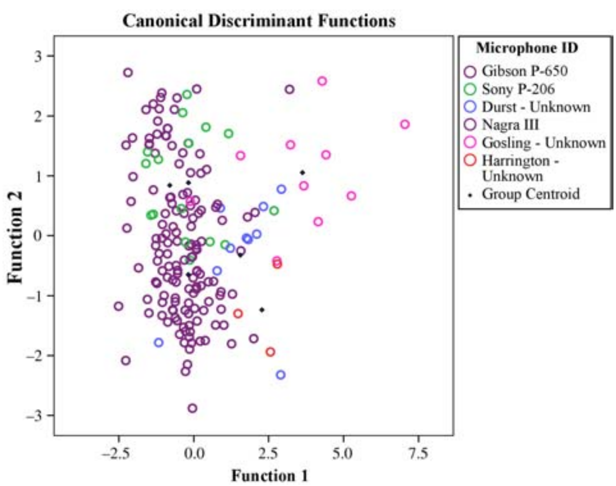

Figure 3. Plot of DFA output for 179 howls recorded with 66.5% accuracy in pack identification, using simple scalar variables. Note the similarity to Figure 2 in the distribution of wolves and that pack accent is weak compared to individual identification.

Discussion

We show that wild Eastern wolves can be individually identified with high accuracy using methods of howl extraction and analysis developed for captive Eastern wolves by Root-Gutteridge et al. (2013). Our findings improve upon other methods (Tooze et al. 1990; Passilongo et al. 2012), with DFA from histogram-derived PCA values for F_0 alone achieving 100% accuracy for wolf identity from solo howls (Table 2).

Normalized harmonic amplitudes were shown to improve individual identification accuracy of howls from wild wolves in their natural habitat, as it was for captive Eastern wolves (Root-Gutteridge et al. 2013). It is likely that by including amplitudes in analyses of other canids, individual identification accuracy in these species may also be improved. We further suggest that the simple scalar variables used in previous bioacoustics studies to accurately assign wolf identity (Tooze et al. 1990; Palacios et al. 2007; Passilongo et al. 2012) can be improved by using DFA with histogram-derived PCA values.

Furthermore, utilization of the new bespoke Matlab extraction code overcame the problem of reliably extracting amplitudes. This has formerly beset *in situ* recording studies (e.g. Frommolt et al. 2003; Mitchell et al. 2006) due to the difficulty in reliably excluding background noise. The new bespoke Matlab extraction code substantially alleviates this difficulty and may allow recordings to be re-analysed with amplitude data included, thereby improving accuracy of identification of individuals from their vocalizations.

The application of encoding individual identity from amplitudes *in situ* requires more work to establish the rate of attenuation over distance, through different habitats and under different atmospheric conditions, and how far this is affected by individuals, either actively or through vocal tract differences. For our data, non-standard recording conditions including distance between recording equipment and wolf did not prevent us from accurately identifying individuals and correctly classifying howls, suggesting that differences in weather conditions and distance to howl will not prevent our method from working. Nevertheless, more detailed analysis of wolf howls in natural habitats is suggested to assess the rate of loss of amplitude across the lowest four harmonics and the effects of distance and weather, with changes in temperature and wind speed expected to have the largest impact on amplitude attenuation (Bradbury and Vehrencamp 1998).

Our findings showed that not all of the amplitude variables were of equal value in identifying individuals, and changes in amplitude of harmonic 3 showed the greatest individuality, contributing the most to correct classification. Mitchell et al. (2006) suggested that coyotes (*Canis latrans*) may control amplitudes of vocalizations in order to achieve the highest fidelity at distances of over 1 km. Whether wolves do the same is unknown but our findings suggest a field of further study, with a focus on whether there is a specific quality of the amplitude of harmonic 3, which clearly carries more information on the individual animal than the other harmonics.

Including all 179 howls, where a wolf could be represented by a single howl, in the analysis produced more tentative findings (82.7–97.4% accuracy, Table 2) than when only the 67 howls from the ten individuals were included in the analysis (88.1–100% accuracy, Table 2). Therefore, it was easier to separate a few wolves represented by multiple howls than many wolves each represented by one or two howls. However, the finding for the full 119 is still the highest accuracy of individual identification for wild wolves using F_0 alone. Furthermore, the complicated chorus howls and low quality of the recordings did not prevent high accuracy of identification of all wolves. Being able to include chorus howls in acoustic analyses improves the usefulness of our method of individual identification as wolves are displaying individuality as part of a group as well as when howling solo

(Theberge and Falls 1967; Palacios et al. 2007). Again, our new method of extraction and analysis could allow howl recordings to be re-analysed to include individual information from chorus howls.

As microphone technology has advanced, it is possible that differences in equipment used to collect howls and the associated differences in recording fidelity (particularly in amplitude) would affect the accuracy of individual identification. Overall, there were differences between the newer microphones and the oldest (Nagra III), with only the two newest microphones achieving 100% accuracy when using F_0 and amplitudes together. However, when only the best quality howls were used, 100% accuracy was still achieved (Table 2). This either indicates that there is no difference in recording quality with different microphones or, more likely, that the new bespoke Matlab code is capable of extracting howl data with minimum influence of microphone type as presumably poorer quality microphones would just produce poorer quality recordings akin to those with lots of background noise.

Classification of individuals (Figures 2 and 3) did not show a clear effect of pack or microphone conclusively as there was some overlay between microphone type and pack identity which could not be separated further. There was also a possibility of regional accent (Figure 3) where wolves from Algonquin Park grouped more closely to each other than wolves from more distant geographic regions. In addition, many of the recordings were from wolves in the same geographic region and therefore probably related to each other (e.g. packs from the 1990s could have been descended from the 1960s), making it impossible to compare pack accent with those found by Passilongo et al. (2010) in Italian wolves. We suggest that both pack and regional accents should be explored in Eastern wolves as they have been in Italian wolves (Passilongo et al. 2010; Zaccaroni et al. 2012), focusing either on differences between packs from the same geographic region in the same time frame or alongside genetic studies to compare potential pack accent with relatedness.

Finally, we suggest that our method of combining the new bespoke Matlab extraction code and improved histogram-derived PCA values could be used in combination with automated howl stimulation boxes (Ausband et al. 2011) which are reusable, movable and reliable for elicited wolf howl recordings to census wolf populations in the future.

Conclusion

The high accuracy of individual identification of captive Eastern wolves from howl recordings (Root-Gutteridge et al. 2013) is repeated here for wild Eastern wolves, suggesting that the new bespoke Matlab extraction code and analysis based on histogram-derived PCA values could improve extraction of vocalizations from recordings of other canid species. This new method of analysis of vocalizations could form the basis of future survey techniques for the individual identification of wild canids.

Supplementary material

Supplementary material for this article is available via the supplementary tab on the article's online page at http://dx.doi.org/10.1080/09524622.2013.817317.

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