1   TITLE

2   Disentangling canid howls across multiple species and subspecies: structure in a complex
3   communication channel

4

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19   WORD COUNT: 5376

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23 ABSTRACT

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25 Wolves, coyotes, and other canids are members of a diverse genus of top predators of considerable

26 conservation and management interest. Canid howls are long-range communication signals, used both

27 for territorial defence and group cohesion. Previous studies have shown that howls can encode

28 individual and group identity. However, no comprehensive study has investigated the nature of

29 variation in canid howls across the wide range of species. We analysed a database of over 2,000 howls

30 recorded from 13 different canid species and subspecies. We applied a quantitative similarity measure

31 to compare the modulation pattern in howls from different populations, and then applied an

32 unsupervised clustering algorithm to group the howls into natural units of distinct howl types. We

33 found that different species and subspecies showed markedly different use of howl types, indicating

34 that howl modulation is not arbitrary, but can be used to distinguish one population from another. We

35 give an example of the conservation importance of these findings by comparing the howls of the

36 critically endangered red wolves to those of sympatric coyotes *C. latrans*, with whom red wolves may

37 hybridise, potentially compromising reintroduced red wolf populations. We believe that quantitative

38 cross-species comparisons such as these can provide important understanding of the nature and use of

39 communication in socially cooperative species, as well as support conservation and management of

40 wolf populations.

41

42 Keywords: Bioacoustics, Coyote, Dog, Howling, Jackal, Social communication, Wolf

1. INTRODUCTION

The genus *Canis* comprises several species and subspecies that share many ecological and behavioural similarities (Bekoff et al. 1981). Most are apex predators, and although some hunt in packs and others alone, all species are strongly social, living in groups ranging in size from a handful of close family members, e.g. coyotes *Canis latrans* (Bekoff 1977), to large groups of 20 or more animals, e.g. Ethiopian wolves *C. simensis* (Sillero-Zubiri & Gottelli 1994). For humans, one of the most familiar canid behaviours is the howl, a long-range communication channel (i.e. a mode through which communication can occur) thought to play a role both in territorial advertising and in group cohesion (Theberge & Falls 1967; Harrington & Mech 1979; Harrington 1987). Howling is most familiar in grey wolves *C. lupus* (Harrington et al. 2003), but all species in the genus produce howl-like vocalisations in addition to other, shorter range communication, such as barks, yips, and growls (Cohen & Fox 1976). These diverse short-range vocalisations are thought to mediate much of canid social behaviour (Yin & McCowan 2004; Siniscalchi et al. 2008), such as maintaining dominance relationships, but it has been speculated that howling too plays a role in inter- and intra-group dynamics (Harrington & Mech 1979; Jaeger et al. 1996; Gese 2001). Support for this hypothesis includes recent studies showing that wolves recognise the howl vocalisations of familiar individuals, and that howls show affective changes in response to the removal of individuals from the group (Mazzini et al. 2013; Palacios et al. 2015). In addition, individual differences in howls have been found in other canid species, e.g. coyotes (Hallberg 2007), and dingoes (Déaux & Clarke 2013).

Canids of all species pose a number of management and conservation challenges. As apex predators, canids have a major influence on prey populations, and changes in canid numbers can result in trophic cascades (Elmhagen & Rushton 2007; Beschta & Ripple 2009). Some species, such as the Ethiopian wolf (Sillero-Zubiri & Gottelli 1994) and the red wolf *C. rufus* (Paradiso & Nowak 1972) are critically endangered, whereas the grey wolf *C. lupus* is frequently in conflict with human populations due to livestock depredation (Sillero-Zubiri & Laurenson 2001), and golden jackals *C. aureus* and domestic dogs *C. familiaris* are considered to be significant reservoirs of rabies (Seimenis 2008; Davlin & VonVille 2012). Management of these issues requires an in-depth understanding of the

71 behavioural ecology of these species and subspecies, which would appear to be incomplete without an

72 understanding of the role of long-range vocal communication. In addition, phylogenetic relationships

73 in the genus as a whole are unclear (Bardeleben et al. 2005; Koepfli et al. 2015), with most

74 component species being capable of producing fertile hybrids, and there is considerable lack of

75 agreement over the status of several grey wolf subspecies and populations (Chambers et al. 2012). As

76 a result, the possible role of vocal behaviour as an isolating factor (or otherwise) between populations

77 is important for the conservation of genetic diversity in subspecies that, while genetically compatible,

78 maintain considerable phenotypic adaptation to their local habitats (Chambers et al. 2012).

79 Partly because of the lack of agreement on the taxonomic status of many canid species and

80 subspecies, and partly for reasons of simplicity, in this paper we will use the term "species" as a

81 shorthand for "species and subspecies".

82 Early studies of canid howling behaviour emphasised qualitative descriptions of howl types

83 (McCarley 1975; Cohen & Fox 1976; Tembrock 1976; Lehner 1978) and overall acoustic

84 characteristics, such as mean fundamental frequency and frequency range, as well as modulation

85 shape measures (Theberge & Falls 1967; Tooze et al. 1990). Multiple variables describing changes in

86 the frequency and amplitude of the howl over time can be used for individual discrimination, among

87 which important discriminative variables are the mean, maximum, and coefficient of variation of the

88 fundamental frequency, and the amplitudes of the various harmonics (Root-Gutteridge et al. 2014a;

89 Root-Gutteridge et al. 2014b). However, there are reasons to consider that information exists in the

90 precise frequency modulation of wolf howls, as well as in simpler acoustic characteristics. Firstly,

91 howls are predominantly narrow-band vocalisations, meaning that most of the acoustic energy is

92 concentrated at a small range of frequencies at any one time. Further, this well-defined frequency

93 varies throughout the course of the howl (Figure 1). This "frequency modulation" is known to be used

94 to encode information in other species with similar vocalisations; particularly bottlenose dolphins

95 *Tursiops truncatus* (Janik & Slater 1998; Quick & Janik 2012)*,* in which individual identity can be

96 reliably extracted from the frequency modulation patterns of whistles (Kershenbaum et al. 2013).

97 Frequency modulation is an effective encoding technique in terrestrial communication (Wiley &

98 Richards 1978), and in addition, considerations of signal transmission indicate that long-range

99   communication in an absorptive environment (e.g. forest) would tend to favour narrow-band

100 frequency modulation over other encoding modalities (Henry & Lucas 2010). Therefore, we consider

101 it appropriate to analyse the frequency modulation of canid howls in a similar way to that of dolphin

102 whistles, to test for characteristic differences between species and populations.

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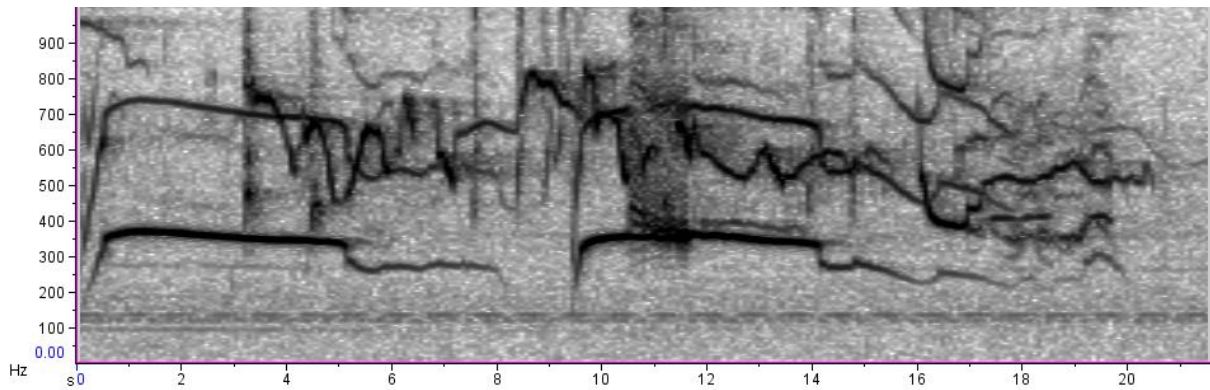
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105 Figure 1. Example spectrogram of multiple wolves howling. The x-axis represents time (seconds) and

106 the y-axis frequency (Hertz).

107

108 A few studies have examined frequency modulation in canid howls, e.g. in coyotes (Hallberg 2007)

109 and Iberian wolves (Palacios et al. 2007), by defining stereotyped modulation patterns such as,

110 "rising", "step down", and "warble to flat". However, these arbitrary categories may not be perceived

111 as distinct units by the focal animal (Kershenbaum et al. 2014), and are potentially subject to selective

112 bias by researchers focusing on "interesting" spectral patterns. Therefore, a thorough analysis of

113 frequency modulation must include (a) a quantitative measure of howl similarity (Deecke & Janik

114 2006), and (b) a quantitative and objective method for grouping howls into distinct howl types,

115 without relying on subjective interpretation. The latter requirement is particularly acute, as a

116 quantitative comparison between the vocal behaviours of different populations is problematic if both

117 repertoires include vocalisations that are qualitatively of a different nature. For example, comparing

118 the howls of one population to the barks of another would be an unproductive effort. Therefore, an

119 alternative paradigm is required that takes into account the partitioning of a vocal repertoire into

120 distinct types, whether arising from functionally different mechanisms (such as howls and barks), or

121 whether being discrete variations of the same functional mechanism (such as different notes in a bird

122 song). We propose that, where multiple distinct vocalisation types are used with overlapping

123 repertoires between populations, the only meaningful way to compare behaviour is to compare the

124 vocalisation type histograms, rather than compare the individual vocalisations. This approach has also

125 been carried out in previous studies of birdsong syntax (Jin & Kozhevnikov 2011). In essence, we

126 interpret the howl type usage histograms as a "fingerprint" of vocal behaviour.

127 In this work, we define and implement a howl similarity metric, as well as an automated clustering

128 technique, and analyse a large database of over 2,000 howls from 21 different species of canids. We

129 classify these howls into distinct types, and compare the relative use of this global repertoire by

130 different populations, thereby testing for objective differences that distinguish between different

131 species. Our results show a diversity of different howl types between species and, although we do not

132 explicitly test for contextual reference in canid howling, we cannot exclude the possibility that

133 specific howl types may be more common in some behavioural contexts than others.

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135

136 2.   METHODS

137

138 We collected a database of canid howling recordings from a wide range of sources. Altogether, we

139 collected 6,009 howls from 21 distinct species, from 207 sources. Recordings were made both of

140 captive and wild animals. The number of sources for each species varied from one (dingo *C. lupus*

141 *dingo* or *C. familiaris dingo*, status unclear, Tibetan wolf *C. l. chanco*, and others) to 20 (eastern

142 timber wolf *C. l. lycaon*). However, we excluded all species with only a single source to avoid

143 confounding individual distinctiveness with species distinctiveness, providing a dataset with 13

144 distinct species from 131 sources. Of these, 2,005 howls were considered to be of sufficient quality

145 for further analysis (no overlapping howls, sufficient signal strength). A breakdown of the recordings

146 is given in Table 1. For each howl, we traced the frequency modulation using a combination of

147 manual and automatic extraction tools, using an image-processing ridge tracker (Kershenbaum &

148 Roch 2013), or by fitting the harmonic peaks to a Lorentzian function (Root-Gutteridge et al. 2014b).

149 Each analysis was reviewed by both AK and HRG for validation.

150

151 Table 1. Number of howls, and number of recording sources (packs) for each of the species in the

152 database.

Canid species	Common name	Reference	Number of howls	Number of sources
C. aureus	Golden jackal		28	3
C. latrans	Coyote		187	4
C. rufus	Red wolf	(Chambers et al. 2012)	79	4
C. lycaon or *C. l. lycaon*	Eastern wolf	(Chambers et al. 2012)	510	20
C. lupus	Grey wolf			
C. l. occidentalis	Mackenzie Valley wolf	(Chambers et al. 2012)	127	8
C. l. baileyi	Mexican wolf	(Chambers et al. 2012)	31	2
C. l. arctos	Arctic wolf	(Chambers et al. 2012)	26	7
C. l. lupus	European wolf	(Nowak 1995)	65	13
C. l. signatus	Iberian wolf	(Vilà et al. 1999)	25	3
C. l pallipes	Indian wolf	(Nowak 1995)	175	7
C. l. lupaster	North African wolf	(Rueness et al. 2011)	33	5
C. familiaris or *C. l. familiaris*	Domestic dog (as companion animal)		375	53
C. hallstromi or *C. l. hallstromi*	New Guinea singing dog	(Koler-Matznick et al. 2003)	344	2

153

154

155 Once the frequency modulation of the howls had been recorded, we compared every howl pairwise to

156 generate a 2,005 x 2,005 matrix of howl similarity/dissimilarity. We used dynamic time warping

157 (DTW) (Kruskal 1983) to deliver a quantitative metric of this distance (or dissimilarity) between

158 every pair of howls. Dynamic time warping has been widely used for comparing frequency data such

159 as these, particularly in the analysis of dolphin vocalisations (Buck & Tyack 1993; Deecke & Janik

160 2006; Sayigh et al. 2007). The DTW algorithm allows the time points of each sampled frequency

161 measurement to vary freely, until an optimum match between the two curves is achieved. The amount

162   of time-axis distortion necessary to achieve this match is then taken as a measurement of the

163   quantitative difference between the curves.

164   Using the dissimilarity matrix obtained by DTW, we applied the k-means unsupervised clustering

165   algorithm to group the howls into natural clusters based on their similarity. We chose the appropriate

166   number of clusters using a combination of cluster purity, measured as the mean cluster silhouette

167   value (Rousseeuw 1987), and stability using a bootstrap technique; repeatedly applying the clustering

168   to a random subset of 90% of the howls, and measuring similarity of the clustering results using

169   normalised mutual information (Zhong & Ghosh 2005).

170   We then examined the usage of each of the different howl types by the 13 different species. As

171   multiple recordings were obtained from the same individual, or from individuals within the same

172   pack, potential issues of pseudoreplication arise if howls are directly compared to each other; howls

173   from the same individual are likely to be more similar to each other than howls from separate

174   individuals or from different packs. Therefore, rather than analysing howl difference distributions

175   directly, we examined only differences in the use of different howl types, by calculating the

176   proportion of howls $P(t)$ that belong to each howl type t, for each species: $P(t)=n(t)/N$, where $n(t)$ is

177   the number of howls of type t for a particular species, and N is the total number of howls from that

178   species. This provides a "fingerprint" of howl type usage, which can then be compared between

179   species. We calculated the sum of squared differences Δ between the howl type histograms of

180   different sources (packs) within each of the 13 species:

$$\Delta(a,b) = \sum_{t}^{T} \left(P_a(t) - P_b(t)\right)^2$$

181   where $\Delta(a,b)$ is the sum of square differences between sources a and b, $P_a(t)$ and $P_b(t)$ are the

182   incidences of howl type t in sources a and b respectively, and T is the total number of distinct howl

183   types for this species.

184   To test the ability of the howl type usage fingerprint to identify canid species, we measured the sum of

185   squared differences Δ between each source (pack) and the mean histograms of each of the 13 species

186   (with the target source excluded), and recorded which species was most similar to the target source as

187 indicated by the lowest value of Δ. From this we constructed a confusion matrix showing the

188 classification of each of the sources, whether to the correct species type or to an incorrect species.

189 We then used an exact test (Fisher 1925) to estimate the significance of the similarity within a species.

190 We randomised the howl type distributions 10^5 times within each species by reordering the incidences

191 $P(t=1...T)$ randomly, and recalculated $\Delta'(a,b)$ to generate a null distribution of sum of squared

192 differences. We then calculated the proportion of randomised differences Δ' that were less than the

193 measured intra-species difference Δ. We also identified the most common howl type in each species

194 and examined various exemplar howls of this type, as an illustration of what may be a typical howl

195 type for this species.

196 Finally, we examined more closely the similarity in the howling behaviour of three sympatric species,

197 the red wolf, eastern timber wolf, and coyote. Red wolves and coyotes hybridise in the wild, which

198 poses a threat to reintroduction programs for the critically endangered red wolf (Hinton et al. 2013;

199 Gese et al. 2015). The eastern timber wolf *C. l. lycaon*, is considered a subspecies of grey wolf, but

200 whose taxonomic status is unclear, and is considered to be very closely related to *C. rufus*, if not

201 conspecific (Wilson et al. 2000; Koblmuller et al. 2009; Chambers et al. 2012). We tested for

202 significant differences between the howls of these three species, to determine whether howling

203 behaviour may potentially provide a form of behavioural isolation, or alternatively encourage

204 admixing and introgression. We reclustered the DTW data, using only howls from the red wolf,

205 coyote, and eastern wolf. We then repeated the sum of square difference analysis, comparing the red

206 wolf-coyote-eastern difference to a null distribution generated by randomising the order of the

207 histogram of howl types, as well as comparing the histogram fingerprints between sources, as with the

208 full data set.

209

210

211 3.   RESULTS

212

213   Applying multidimensional scaling (Cox & Cox 2000) to the full 2,005 x 2,005 matrix of howl

214   distances found 37 significant dimensions, which were then passed to the k-means clustering

215   algorithm. Analysis of silhouette values in k-means led to 21 distinct clusters (howl types). Figure 2

216   shows the howl distance matrix reduced to two dimensions (for visualisation), with cluster assignment

217   indicated. The clustering appeared robust; 99.3% of all howls were classified with posterior

218   probability > 0.5. Bootstrapping and re-clustering with 90% of the data produced a normalised mutual

219   information in comparison to the full data set of 0.760 ± 0.033, i.e. 76% of the cluster assignment

220   information was retained even when applying the algorithm to a reduced data set.

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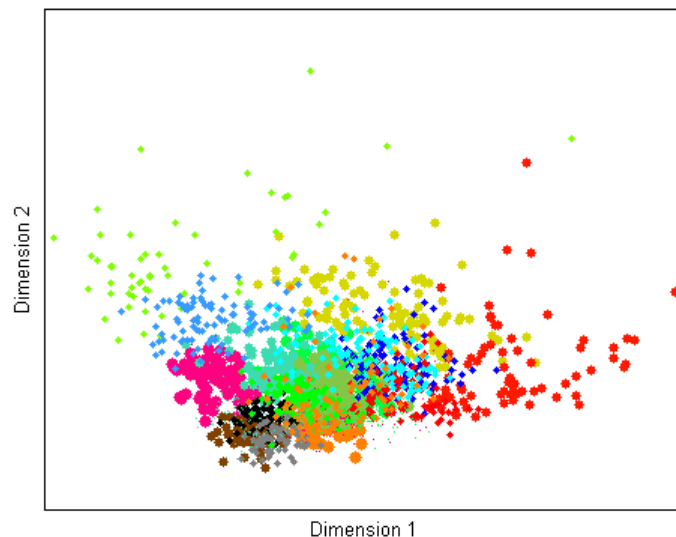
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223   Figure 2. Multidimensional scaling of the 2,005 x 2,005 howl distance matrix into two dimensions.

224   Each point is a howl, and points closer together are more similar than those further apart. Colours

225   indicate k-means clustering assignment. The size of each point is for ease of visualisation only.

226

227   Within-species comparisons show that for the eastern timber wolf, the domestic dog, the coyote, the

228   red wolf, the North African wolf *C.l. lupaster*, and the Arctic wolf *C. l. arctos*, howl type usage was

229   more similar among sources of that species than would be expected by chance (Table 2). This

230   indicates that in these species, the different sites from which recordings were taken showed a species-

231   specific pattern of howl type usage.

232

233 Table 2. Exact test of similarity of howl type use within each species. The p-value represents the

234 proportion of randomised trials where the mean difference between sources within a particular species

235 was less than the actual mean difference within the species. Starred values are significant at 5%.

| Species	p		Number of sources
Golden jackal	0.718		3
Coyote	0.019	*	4
Red wolf	0.007	*	4
Eastern Timber wolf	0.014	*	20
Mackenzie Valley wolf	0.955		8
Mexican wolf	0.891		2
Arctic wolf	0.006	*	7
European wolf	0.237		13
Iberian wolf	0.935		3
Indian wolf	0.144		7
North African wolf	<0.001	*	5
Domestic dog	0.003	*	53
New Guinea singing dog	0.899		2

236

237

238 The use of each howl type, adjusted for overall howl use frequency, for each of the species show

239 species-specific fingerprints (Figure 3). The red wolf and coyote share howl type 3 as the most

240 common; the European *C. l. lupus* and Iberian *C. l. signatus* wolves share type 5; and the Mackenzie

241 Valley *C. l. occidentalis*, Indian *C. l. pallipes*, and Mexican *C. l. baileyi* wolves share type 7. Each

242 other species has a distinct call type that is most commonly used, relative to its overall usage in the

243 sample database. Apart from these distinctive howl types, the different species have different

244 repertoire diversities, with for instance the North African wolf making use of many fewer howl types

245 than the golden jackal, despite being represented by a similar overall number of sources and howls

246 (Figure 3). One qualitative trend noticeable from the exemplar howls (chosen as those nearest to the

247 cluster centroid) is that the smaller species (red wolf, coyote, New Guinea singing dog, domestic dog,

248 golden jackal) favoured howls that ended with a sharp drop in frequency, whereas larger species

249 (arctic wolf, eastern timber wolf, European wolf, Mackenzie Valley wolf) used howls with much less

250 frequency modulation, particularly at the end of the howl (Figure 4), although this may be an artefact

251 of the lower fundamental frequency used by larger species.
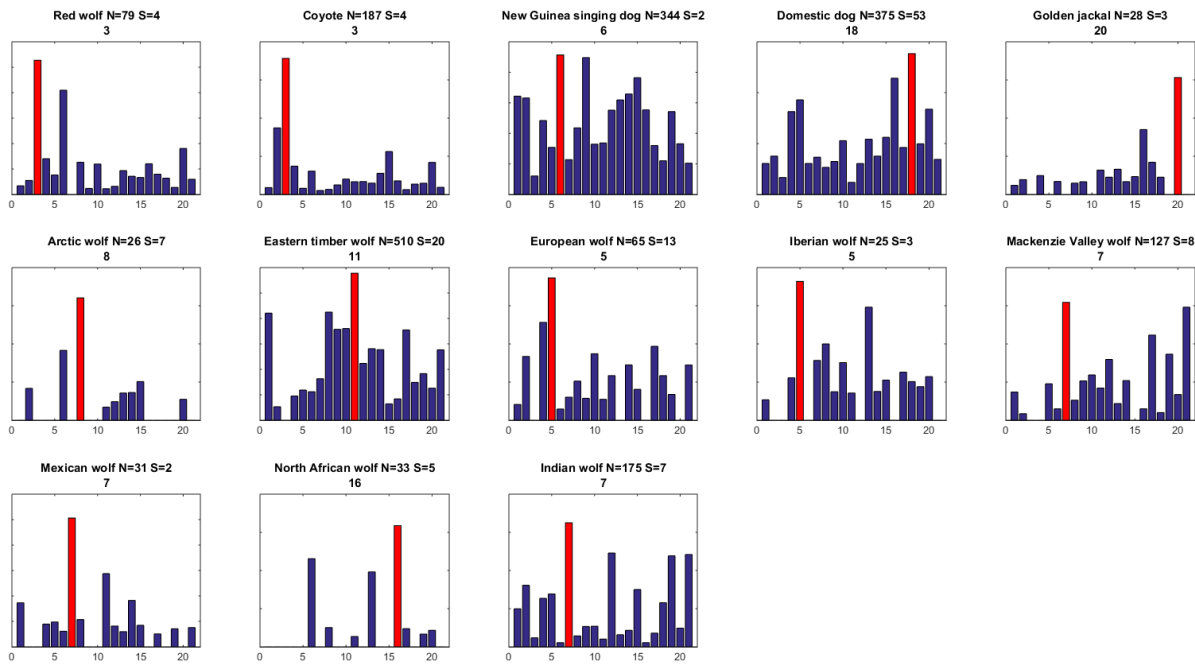
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Figure 3. Howl use histograms for each of the 13 species, showing the relative use of each of the 21 howl types, adjusted for overall howl type frequency. Red bars show the most commonly distinctive howl type for each species, with the index number of that type appearing above each histogram. *N* indicates the number of howls, and *S* indicates the number of sources.

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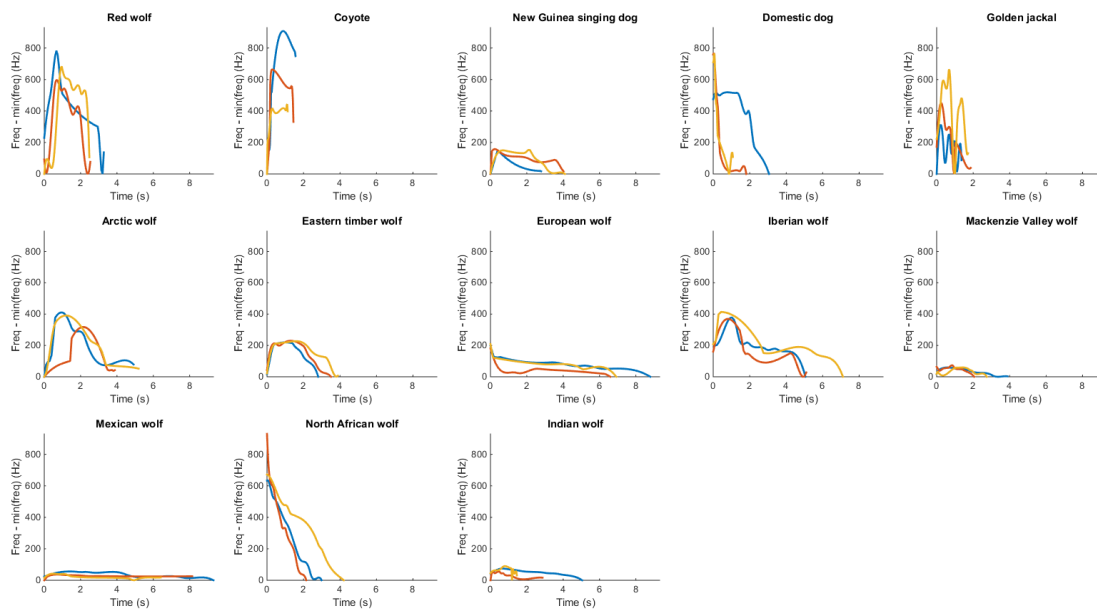
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260   Figure 4. Three examples of the howls of the particular howl types identified as characteristic of each

261   species, and represented in Figure 2 by the red bars. Note that the howls within a type are similar in a

262   dynamic time warping sense, although they may vary somewhat in length.

263

264

265   The confusion matrix for the identification of species by source, and the results of the species

266   identification assessment (Table 3) shows that the coyote, Arctic wolf, and North African wolf all

267   were well identified by howl usage fingerprint comparison, with identification of the red wolf and

268   Mackenzie Valley wolf also higher than expected. The New Guinea singing dog *C. l. hallstromi*,

269   domestic dog, golden jackal, and North African wolf appeared to form a cluster of similar howl usage

270   types, and the coyote and red wolf seem to form a separate cluster, with heavy use of type 15 howls

271   (which only seem to be used by 3 other species, and at very low frequency).

272

273   Table 3a. Classification success by comparing howl type usage histograms as fingerprints. The %

274   correct column indicates how many recording sources (animal packs) were correctly identified as their

275   particular species when compared to all other sources in the database. The Best guess column

276   indicates which species were most frequently identified as the most similar species to the target

277   source.

| Species	% correct	Best guess
Golden jackal	33.3	Domestic dog
Coyote	50	Coyote
Red wolf	25	Red wolf, Coyote, Domestic dog, Arctic
Eastern Timber	5	Arctic
Mackenzie Valley	25	Mackenzie Valley, Indian
Mexican	0	Red wolf
Arctic	57.1	Arctic
European	0	Mackenzie Valley
Iberian	0	Eastern Timber , European , Mackenzie Valley
Indian	0	Mackenzie Valley
North African	40	North African
Domestic dog	13.2	North African
New Guinea Singing Dog	0	Domestic dog, North African

278

279   Table 3b. Confusion matrix showing the number of sources identified as each species type.

	Predicted species												
Actual species	RW	COY	NGSD	DD	GJ	ARC	ETIM	EUR	IBER	MV	MEX	NAFR	IND
RW	1	1		1		1							
COY	1	2									1		
NGSD				1								1	
DD	3	3	1	7	4		3	8	3	2	2	9	8
GJ				2	1								
ARC		1	1			4							1
ETIM	3					5	1	1	3	4			3
EUR		2		2		2			2	3	1		1
IBER							1	1		1			
MV	1			1			1	1		2			2
MEX	1								1				
NAFR	1		1		1							2	
IND		1		1				1		4			

281

282   In the reduced analysis of just red wolf, coyote, and eastern timber wolf, there were a total of 776

283   howls, 510 eastern timber wolf, 187 coyote, and 79 red wolf. Applying multidimensional scaling to

284   the full 776 x 776 matrix led to 42 significant dimensions, and 11 k-means clusters. All howls (100%)

285   were classified with posterior probability > 0.5, and bootstrapping followed by reclustering led to a

286   normalised mutual information of 0.706 ± 0.059. With these data, the red wolf and coyote also

287   showed significant similarity between the different sources (packs) of the same species (p=0.006 and

288   p=0.009 respectively), whereas the eastern timber wolf was only marginally significant (p=0.052).

289   Comparison of the howl type fingerprints showed that the coyote was well identified from most

290   sources (3 out of 4 sources correctly identified), whereas the red wolf and eastern timber wolf were

291   often misclassified one as the other, with the red wolf identified as eastern timber wolf in 2 out of 4

292   sources, and eastern timber wolf as red wolf in 6 out of 20 sources. Red wolves and coyotes share

293   their most common howl type – type 3 – which is rarely used by timber wolves. Red wolves will often

294   use howl type 6, which coyotes and timber wolves rarely use, and may be intermediate in

295   characteristics between coyote (type 3) and timber wolf (type 11) howls, by being lower in frequency

296   and less frequency-modulated (Figure 5).
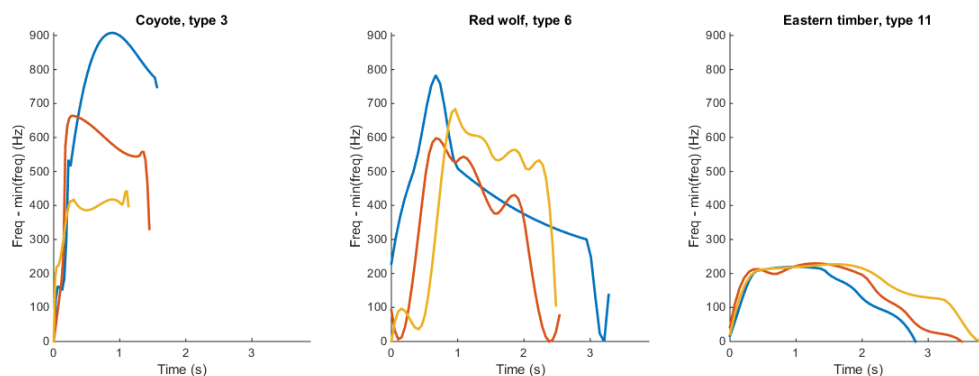
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298

299   Figure 5. Examples of coyote howls of type 3 (left), red wolf howls of type 6 (middle) and eastern

300   timber wolf howls of type 11 (right). Type 6 howls are rarely used by coyotes and eastern timber

301   wolves, but commonly used by red wolves, and may represent an intermediate form.

302

303

304   4.   DISCUSSION

305

306   In this study we analysed over 2,000 howls from 13 different species and subspecies belonging to the

307   genus *Canis* around the world. Using dynamic time warping as a quantitative measure of howl

308   dissimilarity, we applied an objective unsupervised clustering algorithm to group the howls into

309   distinct howl types. The k-means algorithm produced 21 clusters that were stable to bootstrapping,

310   and that probably represent genuine howl type categories, which we define without the need for

311   subjective description of howl characteristics.

312   Each population recorded made different use of these 21 howl types, with many species/subspecies

313   having a particular howl type that was characteristic of that species/subspecies. Within six of the

314   species - the eastern timber wolf, the domestic dog, the coyote, the red wolf, the North African wolf,

315   and the Arctic wolf - a statistically significant similarity existed in their howl type usage. Further, we

316   found that individual populations of five of the species - coyote, red wolf, Arctic wolf, North African

317   wolf, and Mackenzie Valley wolf - could be identified using the howl type histograms of the

318   remaining populations in the data set.

319   In general, we conclude that canid howling is not an arbitrary signal, but possesses species-specific

320   information, which may reflect adaptive and/or neutral processes of isolation.

321   We also performed a more detailed analysis of the howls of three American canids - the red wolf, the

322   coyote, and the eastern timber wolf - because of the conservation importance of hybridisation between

323   the critically endangered red wolf and the coyote, as well as continuing disagreement over the

324   phylogenetic relationship between the red wolf and both the coyote and eastern timber wolf. We

325   found that coyote and red wolf howl type usage differs significantly, which could be a useful tool for

326   managing red wolf conservation in the face of competition from sympatric coyotes. Red wolf howling

327   was similar to that of eastern timber wolves, further complicating the challenge of red wolf

328   introduction both at the southern end of its range (coyotes) and at the northern end (eastern). In

329   contrast, red wolves and coyotes share their most common howl type, whereas red wolves will often

330   use howl type 6, which coyotes and timber wolves almost never use. The intermediate nature of howl

331   type 6 may provide potential evidence of hybridization between these sepecies.

332   We note in passing that the smaller species - the red wolf, domestic dog, New Guinea singing dog,

333   golden jackal - show a greater diversity of howl types than the larger species, and are similar to each

334   other in their howl type usage. We lack sufficient data to examine this further; however, this

335   phenomenon could be due to peculiarity of the habitat or niche of these smaller species, or could be

336   due to a different emphasis on long and short range communication between larger and smaller

337   species, differences in the vocal production mechanism in different sized species, or a different

338   emphasis on the social role of howling.

339   Given the diverse and non-arbitrary nature of howl differences, it is natural to ask whether variations

340   in howl structure reflect referential or context-specific information. Early studies of wolf

341   communication pointed out that different vocalisation types (e.g. howl vs. growl, yelp, etc.) were

342   associated with different behavioural contexts (Cohen & Fox 1976; Tembrock 1976), but stopped

343   short of suggesting that particular features within howls themselves represented certain arousal states

344   or environmental contexts (Theberge & Falls 1967; Lehner 1978). More recent studies have begun to

345   address this question in dingoes (Déaux & Clarke 2013), as well as dogs (Faragó et al. 2014), and

346   there is some evidence that vocal communication may be used in *Canis* to coordinate hunting activity

347   (Muntz & Patterson 2004). Experimentally, it has been shown that howl modulation patterns convey

348   individual identity, and that animals attend to this information (Palacios et al. 2015). Thus, individual

349   identity in howl structure is more than just an epiphenomenon, and may be of relevance to

350   conservation and management programs (Llaneza et al. 2005; Terry et al. 2005; Brennan et al. 2013;

351   Hansen et al. 2015). Depredation of livestock by coyotes (Knowlton et al. 1999) and wolves (Sillero-

352   Zubiri & Laurenson 2001), in particular, is a cause for concern, but attempts to use vocalisation

353   playbacks as active deterrents have largely been unsuccessful (Gable 2010).

354   Our results have shown clear differences in howl structure between populations. Whether populations

355   in geographical proximity represent separate species, subspecies, or otherwise, it seems clear that

356   distinct ecotypes exist. The presence of discrete differences in vocal behaviour suggests that

357   consideration should be given to conservation of populations such as *C. rufus* and *C. lupus lycaon*,

358   even if genetic isolation does not exist. Recent studies have shown multiple examples of dialects not

359   just in birdsong (Kroodsma 2004), but also in multiple mammalian taxa including rodents

360   (Slobodchikoff & Coast 1980; Gannon & Lawlor 1989), primates (de la Torre & Snowdon 2009;

361   Thinh et al. 2011; Meyer et al. 2012), and hyraxes (Kershenbaum et al. 2012). Our study adds to

362   recent work showing dialectic differences between the howls of wolves in Europe and North America

363   (Palacios et al. 2007), and fits into an increasingly important trend of understanding the proximal

364   causes and ultimate significance of dialectic variation (Lameira et al. 2010).

365   In the case of the critically endangered red wolf, hybridisation with coyotes represents the largest

366   threat to reintroduced populations (Hinton et al. 2013; Gese et al. 2015). Although howling behaviour

367   has long been identified in *Canis* as a mechanism for separating competing populations (Harrington &

368   Mech 1979; Jaeger et al. 1996; Gese 2001), and vocal behaviour as a mechanism for genetic isolation

369   in other mammalian taxa (Braune et al. 2008), to our knowledge no studies have addressed the

370   question whether vocal differences can act to reduce interspecific hybridisation in *Canis*, or may in

371   fact be the result of past hybridisation. Coyotes fail to respond to stimuli of wolf howling (Petroelje et

372   al. 2013); detailed analysis of *C. rufus* recordings have uncovered non-howl vocalisations that have

373   not been reported in *C. latrans* (Schneider & Anderson 2011); and the behavioural responses of

374   individual wolves vary according to the familiarity of playback howls (Palacios et al. 2015). All these

375   findings raise the possibility that vocal differences between *C. rufus* and *C. latrans* may have

376   conservation significance. Our work adds to this body of evidence, and should encourage further

377   investigation of the possibility of behavioural isolation between these populations.

378   Our study made use of data sources of widely varying size and quality - something inevitable when

379   integrating recordings from around the world and from species of greatly varying abundance. We

380   have endeavoured to minimise the statistical artefacts arising from this imbalance, and have been

381   careful to use the recording source (essentially, a single pack) as the unit of comparison. Some

382   pseudo-replication may remain, as we cannot ensure that the proportion of howls in each type is

383   constant for a species. However, in most cases there are insufficient howls from specific individuals to

384   look at how the pattern varies by individual within species. In addition, it is possible that differences

385   between populations reflect differences in contextual stimuli. Despite these statistical limitations, we

386   believe that such broad comparative studies have great value in understanding behaviour across a

387   wider taxonomic basis than just the species, and we hope that this utility compensates somewhat for

388   the patchy nature of the data sources.

389   Automatic clustering using unsupervised algorithms is potentially problematic, as the presence of

390   computer-identified clusters does not guarantee that these elements have cognitive significance for the

391   animals involved. Indeed, we have no mechanistic indication that canids perceive and compare howls

392   in a way similar to our dynamic time warping. To date, what we know is that wolves detect changes in

393   the fundamental frequency of howls outside their natural range of variability, and changes in the

394   frequency modulation pattern of howls (Palacios et al. 2015). However we feel confident that DTW

395   provides a useful comparative tool, because consideration of acoustic propagation would indicate that

396   frequency modulation of howls is likely an important encoding technique in long-range

397   communication. Also, we took care to evaluate our clustering results using multiple metrics, and

398   assessing their stability in the face of bootstrapping, to maximise confidence that the howl type

399   partitions did, in fact, represent a division of howls into realistic howl types.

400   This study has involved only correlative analyses, but we believe that this kind of quantitative

401   categorisation of vocalisation types is necessary before carrying out manipulative and playback

402   experiments. Being armed with an objective set of howl types, or a methodology for arriving at such a

403   definition, allows researchers to test the cognitive significance of different howl compositions, and

404   look for potential behavioural correlates, such as territorial advertising and group cohesion. Any

405   experimental work with critically endangered species such as the red wolf can be problematic, but we

406   hope that with a firmer understanding of the vocal behaviour of these animals, it will be possible to

407   design experiments that will benefit the conservation and management of this and other species.

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410   5.   CONCLUSIONS

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412   Howling is a social communication process that is likely of major importance in the overall behaviour

413   of all canid species. A deeper understanding of their social behaviour is not possible without a

414   framework within which to understand their vocal behaviour. In particular, quantitative and objective

415 assessment of howling is highly preferable to subjective interpretation by humans, who lack the

416 auditory and cognitive instruments of the focal animals. Techniques such as ours providing

417 quantitative comparisons are important for any future experiments to investigate functionally

418 referential elements to the canid howl repertoire, which would be a highly significant finding for two

419 reasons. Firstly, the role of vocal communication in mediating social behaviour in canids may

420 contribute to understanding the evolution of human language (Seyfarth & Cheney 2014). To our

421 knowledge, no animal species other than humans possess any form of true language, not even any

422 form of "proto-language". Therefore, it has been problematic to explain the evolution of human

423 language as a continual progression from "non-language" to "language", through increasing adaptive

424 advantage at each step (Tomasello 2008). The presence of complex referential communication in

425 species that must cooperate to survive was probably a crucial step in the evolution of language

426 (Jackendoff 1999). Understanding the communication systems of extant social species is essential to

427 understanding the potential evolutionary trajectories to more complex communication that have

428 occurred in the past, eventually leading to human linguistic abilities. Although taxonomically distant

429 from early hominids, wolves and other canids show remarkable parallels with humans in, for example,

430 social behaviour, intelligence, and vocal communication (Miklósi 2014). Further understanding of

431 canid social communication could serve as a model system for the evolution of more complex vocal

432 communication and language. Secondly, canid conservation and management can benefit from

433 acoustic methods for surveying and assessing population size and health/genetic purity (Llaneza et al.

434 2005; Brennan et al. 2013), which can be difficult using traditional methods, particularly when snow

435 is absent (Blanco & Cortés 2011). Active acoustic deterrence has also been suggested as a tool in the

436 control of animal movements for mitigating wolf conflict with farmers (Gable 2010), but such

437 techniques cannot be successfully implemented without understanding the message being transmitted.

438 Therefore, clearer and quantitative techniques for the description of the long-range communication of

439 canids could have multiple benefits for the conservation and management of these species: through

440 understanding the processes controlling behavioural isolation, identifying populations by remote

441 surveying, and active techniques to reduce human-animal conflict.

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467   7.   References

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641

642

643   FIGURE CAPTIONS

644

645   Figure 1. Example spectrogram of multiple wolves howling.

646

647   Figure 2. Multidimensional scaling of the 2,005 x 2,005 howl distance matrix into two dimensions.

648   Each point is a howl, and points closer together are more similar than those further apart. Colours

649   indicate k-means clustering assignment. The size of each point is for ease of visualisation only.

650

651   Figure 3. Howl use histograms for each of the 13 species, showing the relative use of each of the 21

652   howl types, adjusted for overall howl type frequency. Red bars show the most commonly distinctive

653   howl type for each species, with the index number of that type appearing above each histogram. N

654   indicates the number of howls, and S indicates the number of sources.

655

656   Figure 4. Three examples of the howls of the particular howl types identified as characteristic of each

657   species, and represented in Figure 2 by the red bars. Note that the howls within a type are similar in a

658   dynamic time warping sense, although they may vary somewhat in length.

659

660   Figure 5. Examples of coyote howls of type 3 (left), red wolf howls of type 6 (middle) and eastern

661   timber wolf howls of type 11 (right). Type 6 howls are rarely used by coyotes and eastern timber

662   wolves, but commonly used by red wolves, and may represent an intermediate form.

663

664