

Biphonation May Function to Enhance Individual Recognition in the Dhole, *Cuon alpinus*

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Abstract

Biphonation (two independent fundamental frequencies in a call spectrum) represents one of the most widespread nonlinear phenomena in mammalian vocalizations. Recently, the structure of biphonations was described in detail; however, their functions are poorly understood. For the dhole (*Cuon alpinus*), biphonic calls represent a prominent feature of vocal activity. In this species, the biphonic call is composed of two frequency components – the high-frequency squeak and the low-frequency yap, which also occur alone as separate calls. In this study, we test the hypothesis that the complication of call structure, resulting from the joining of these calls into the biphonic yap–squeak may enhance the potential for individual recognition in the dhole. We randomly selected for analysis 30 high-frequency squeaks, 30 low-frequency yaps and 30 biphonic yap–squeaks per animal from five subadult captive dholes (450 calls in total). Discriminant analysis, based on 10 squeak parameter values, showed 80.7% correct assignment to a predicted individual. For 10 yap parameters, the correct assignment was only 44.7%. However, the analysis based on 10 parameters of the biphonic yap–squeak, selected as best contributing to discrimination, showed 96.7% correct assignment to a predicted individual. The results provide strong support for the hypothesis tested showing that the joining of two independent calls into a common vocalization may function to enhance individual recognition in the dhole.

Introduction

Biphonation is one of the nonlinear phenomena in mammalian vocalizations that evoked a burst of research interest in recent years (Wilden et al. 1998; Riede et al. 2000; Fitch et al. 2002). Biphonation may be recognized: (1) by appearance of two independent fundamental frequencies in a sound spectrum, (2) by different contours of frequency modulation in some frequency bands on a spectrogram, (3) by the appearance of additional frequency bands, representing linear combinations of two independent frequencies, which may be calculated using the formula $n \cdot f + m \cdot g$ (f and g are two independent

frequencies; n and m are integers) (Wilden et al. 1998; Volodin & Volodina 2002). At least four probable mechanisms for production of biphonic vocalizations have been proposed: (1) asynchronous vibration pattern of the left and right vocal fold (Berry et al. 1996; Tigges et al. 1997); (2) involvement of vocal fold extensions (vocal membranes) in the production of a second fundamental frequency (Brown & Cannito 1995; Mergell et al. 1999; Riede et al. 2000); (3) vortex-shedding at the glottal constriction inducing a whistle-like sound (Solomon et al. 1995; Herzel & Reuter 1997; Wilden et al. 1998); and (4) source-tract coupling (Herzel & Reuter 1996; Mergell & Herzel 1997). These proposals

come from three sources: from analysis of actual animal and human calls (Brown & Cannito 1995; Herzel & Reuter 1996, 1997; Tigges et al. 1997; Wilden et al. 1998; Riede et al. 2000), from experiments on excised larynges (Solomon et al. 1995; Berry et al. 1996; Brown et al. 2003), and from computer simulation models (Mergell & Herzel 1997; Mergell et al. 1999). However, research of the role of various mechanisms in production of biphonic calls in particular species is limited, and sound production mechanisms in nonhuman mammals remain poorly understood (Peters et al. 2002).

Functional interpretations of nonlinear phenomena in vocalizations are also scarce, and nearly entirely absent with concern to biphonation. These phenomena are not under central nervous system control, but arise from the physics of a mammalian sound production apparatus and thus may not have an adaptive meaning for a caller (Wilden et al. 1998; Fitch et al. 2002). For example, subharmonics, chaos and biphonations occur in unhealthy voices both in humans (Herzel 1993; Herzel et al. 1995; Herzel & Reuter 1996) and in nonhuman mammals, such as Japanese macaque *Macaca fuscata* (Riede et al. 1997), domestic dog *Canis familiaris* (Riede et al. 2001) and domestic cat *Felis catus* (Riede & Stolle-Malorny 1999).

Although some nonlinear phenomena in voices may not have evolved as a structural feature with a communicative meaning a priori, they may have been adopted subsequently (Fitch et al. 2002). Moreover, there are some data confirming the communicative functions of biphonation. In two penguin species – the king penguin *Aptenodytes patagonicus* and the emperor penguin *A. forsteri* – beating generated by interaction of two close frequencies enhances the ability of calls to propagate through obstacles of numerous penguin bodies in a colony and provides additional cues for both parent–chick and mate–mate recognition (Aubin et al. 2000; Lengagne et al. 2001; Aubin & Jouventin 2002). In the killer whale *Orcinus orca*, differences in degradation and directionality of the lower- and higher-frequency components in biphonic calls provide information about direction of movement of a caller (Miller 2002). In addition, among proposed functions of nonlinear phenomena are accenting call formant structure for delivering information about size of a caller and breaking of monotony in vocal sequences in order to escape habituation in listeners and to attract their attention to a caller (Fitch & Hauser 2002; Fitch et al. 2002).

Biphonic calls have been recorded in a few terrestrial mammalian taxa, and besides such specialized group as bats (Kanwal et al. 1994), they have been

recorded primarily in primates (Brown & Cannito 1995; Brown et al. 2003) and canids (Wilden et al. 1998; Volodin & Volodina 2002). Biphonation has been recorded in timber wolf *Canis lupus* (Nikol'skii & Frommolt 1989; Frommolt 1999), domestic dog (Solomon et al. 1995; Volodin et al. 2005b; Volodina et al. 2005), dog–wolf hybrid (Riede et al. 2000) and jackal *Canis aureus* (A. Pojarkov, pers. comm.), but are especially prominent both in the African wild dog *Lycaon pictus* (Wilden 1997; Wilden et al. 1998; Robbins 2000) and in the dhole (Volodin & Volodina 2002). In canids, biphonic calls may occur as irregular events and not in all individuals, or attend specific states, such as frustration in domestic dogs (Volodin et al. 2005b; Volodina et al. 2005). But, in African wild dogs and dholes, biphonic calls occur regularly among calls attending short-distant affiliative interactions in a pack and make up 60% and 44% of vocal emissions in this context, respectively. Moreover, they occurred in all individuals in these species (Wilden et al. 1998; Volodin & Volodina 2002).

The dhole is a pack-living canid, communally hunting on large prey and inhabiting areas with complex relief in mountains and in locations with dense vegetation, with primary breeding by a dominant pair and other group members functioning as helpers (Cohen 1977; Johnsingh 1982; Karanth & Sunquist 1995; Venkataraman et al. 1995; Venkataraman 1998). Dholes typically show very high vocal activity, that attend all, even small movements of pack members and all contacts among the animals (Sosnovskii 1967; Cohen 1977, 1985; Johnsingh 1982). In captivity, the dhole vocal repertoire includes 11 call types, based on three vocal components: the low-frequency tonal (with fundamental frequency varying from 0.5 to 1.4 kHz), the high-frequency tonal (with fundamental frequency varying from 5.5 to 10.8 kHz), and the pulsed component. Only one call type has a biphonic structure, resulting from simultaneous production of the high- and low-frequency components (Volodin et al. 2001). Biphonic calls (call type yap–squeak), alongside with 'clear' yaps and squeaks and frequency jumps from squeak to yap, occurred primarily during peaceful interactions among group members and in the context of spontaneous movements in an enclosure (Volodin et al. 2001; Volodin & Volodina 2002). In these situations, the occurrence of biphonic calls (yap–squeaks) among contact calls (yaps, squeaks and yap–squeaks) varied in 14 individual dholes from 20% to 92%, and was not related significantly to age, sex or litter membership (Volodin & Volodina 2002).

It is not clear if yap-squeak is the same call type known as 'mixed yip-yack cackle' after Johnsingh (1982). Moreover, it is difficult to relate it to other call types reported onomatopoeically in earlier literature, because of the absence of spectrograms in these papers. Moreover, biphonic calls are difficult to discern from some other call types by ear. Therefore, based on the context of usage of these calls in captivity, we propose their function to be as peaceful vocalizations and that these calls may promote individual recognition in dholes. Furthermore, we propose that the complexity of the call type, composed from two independent frequencies, may play a special role, enhancing reliability of the recognition. Here we test using discriminant analysis, if the biphonic calls, composed of the high- and low-frequency components, provide better potential for individual identification, than the monophonic calls consisting of one of these components.

Animals and Methods

Calls were tape-recorded from five subadult dholes (aged 7.5–11 months) from two litters born in captivity. The first litter of one male and two females (no. 10, 11, 12) was born in March 19, 1999 in Moscow Zoo (Russia). The second litter of three males was born in April 24, 1999 in Volokolamsk Moscow Zoo Brooder, but only two of them (no. 14, 15) provided the necessary number of calls to be included in the analysis. All the recordings were made during November 2, 1999 to February 21, 2000. All the animals were housed with their littermates. Parents were housed together with pups (first litter) or separated from them by wire mesh (second litter).

The sound recordings were made with a SONY WM-D6C recorder (Sony Corp., Tokyo, Japan) and MCE-100 unidirectional microphone (LOMO, St. Petersburg, Russia). Frequency responses of both systems were 40–15 000 Hz. Distance to animals during the recordings varied from 2 to 8 m. The sounds were produced spontaneously without stimulation from observers. All individuals from the same litters could be reliably identified by their coloration pattern. Simultaneous video recordings to identify calling individuals were made with a SONY TRV-65E video camera.

For analysis, we randomly selected 90 high-quality calls per individual (30 yaps, 30 squeaks and 30 biphonic yap-squeaks, 450 calls for five dholes in total). Ownership of each call was confirmed independently by two observers during recordings which

was confirmed additionally on the basis of video recordings.

All spectrograms of these calls were analyzed with Avisoft SASLab software (© R. Specht). Digital processing used fast Fourier transform (FFT) with 22.05 kHz sampling frequency, Hamming window, FFT length 512 points, frame 50%, overlap 93.8%, that provided 1.45 ms time resolution and 43 Hz frequency resolution.

Biphonic calls of dholes comprise two independent vocal components that also occur as separate vocalizations. The fundamental of the low-frequency component f_0 is about 1 kHz, and the fundamental of the high-frequency component g_0 is higher than 5 kHz (Fig. 1). We measured seven frequency and three temporal parameters for each component occurring alone as yap and squeak calls and 20 parameters for each biphonic yap-squeak (Table 1) For the biphonic calls, the high- and low-frequency component parameters were measured after high- and low-pass filtration of 5 kHz, applied alternately. Temporal parameters were measured from the spectrogram window using a standard marker cursor. Fundamental frequency parameters for each component were measured using a free reticule cursor. All measurements were exported automatically into an Excel database. The number of fundamental frequency extrema were counted visually from spectrograms according to Tooze et al. (1990). Frequency parameters of the high-frequency components were measured by g_0 only, whereas those of the low-frequency components by the most well-expressed frequency band (f_0 , f_1 or f_2). Values of peak frequency and bandwidth of peak frequency (at distance -10 dB from peak) of the corresponding component

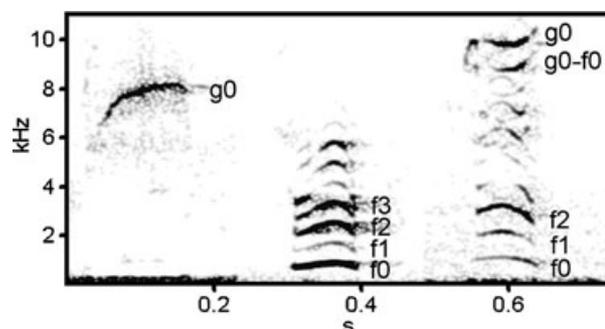


Fig. 1: Spectrograms illustrating three call types of the dhole: left – the high-frequency call or squeak; middle – the low-frequency call or yap; right – the biphonic call or yap-squeak. Designations: g_0 – fundamental frequency of the high-frequency component; f_0 – fundamental frequency of the low-frequency component; f_1 and f_2 – harmonics of f_0 ; g_0-f_0 – the linear combination of f_0 and g_0

Table 1: Call parameters used in the statistical analyses

Call parameters	High-frequency component	Low-frequency component
Start fundamental frequency (kHz)	g0_ini	f0_ini
End fundamental frequency (kHz)	g0_end	f0_end
Maximum fundamental frequency (kHz)	g0_max	f0_max
Minimum fundamental frequency (kHz)	g0_min	f0_min
Peak frequency (frequency with the maximum amplitude in the power spectrum) (kHz)	g_peak	f_peak
Bandwidth of peak frequency (Hz)	g_bandw	f_bandw
Number of fundamental frequency extrema (total number of peaks and depressions)	g_extrem	f_extrem
Duration from start to maximum frequency point of a component (s)	g_dur_inc	f_dur_inc
Duration from maximum frequency point to end of a component (s)	g_dur_dec	f_dur_dec
Proportion: duration from start to maximum frequency point of a component/duration of a component	g_k_max	f_k_max

were taken automatically from the mean power spectrum. For the high-frequency component, the peak frequency band coincided with fundamental frequency g_0 , otherwise, for the low-frequency component, it could fall on one of the harmonic bands from f_0 to f_2 , predominately on f_1 . We also used one calculated parameter: the duration from the start to the maximum frequency point of a component divided by the duration of the component (k_{max}) (Table 1).

For each of the three call types, values were normally distributed for most parameters (Kolmogorov–Smirnov test). As parametrical ANOVA and discriminant analysis are relatively robust to departures from normality (Dillon & Goldstein 1984), this was not an obstacle to the application of these tests.

We performed one-factor ANOVA, with ‘individual’ as the grouping variable, to compare variability of the parameters within and between individuals for each call type. Then we used the standard discriminant analysis procedure based on 10 parameters for each call to determine whether calls could be assigned to the correct caller. Because the biphonic calls contained a double set of parameters, 10 from the low frequency, and 10 from the high frequency, we had to reduce this number to 10 to escape increasing discriminability of biphonic calls simply because of an increase in the number of parameters entered into the analysis. To select 10 of the 20 parameters available for biphonic calls, we conducted a stepwise discriminant analysis and took the 10 parameters that best contributed to the discrimination. For statistical comparison of correct assignment values resulting from discriminant analyses for the biphonic and non-biphonic calls, we used a 2×2 chi-squared test.

To validate results of discriminant analysis, we performed cross-validation analysis and randomization. For cross-validation analysis, call samples for

each dhole were randomly split half-and-half, providing a training set (75 calls) and a test set (75 calls) for each call type. Then the classification of one half of the dataset was made, with the discriminant function derived from the other half.

Randomization was applied in order to calculate the expected level of correct assignment by discriminant analysis if the calls we analyzed were randomly distributed between individuals. For this procedure, we created five randomization groups for each call type. Each group of 30 calls consisted of six randomly selected calls taken from each of five dholes. After that, we conducted a standard discriminant analysis and calculated the probabilities of correct assignment of calls to the randomization groups. These probabilities were taken as random values for each call type. Differences between the random and actual values of correct assignment were tested with a 2×2 chi-squared test. All the analyses were performed in STATISTICA, version 6.0 (StatSoft, Inc, Tulsa, OK, USA).

Results

The one-way ANOVA revealed the highly significant individual differences in all call parameters for individuals both for squeaks (10 parameters) and yap-squeaks (20 parameters), but only for four call parameters of yaps (f_0_{end} , f_0_{min} , $f_{dur_{dec}}$, $f_{k_{max}}$). For the other six call parameters of yaps, interindividual variability did not exceed intra-individual variability. Table 2 shows mean values and standard deviations for some call parameters (for four parameters both for the high-frequency squeak and the low-frequency yap as well as for eight parameters of biphonic yap-squeak calls) and results of ANOVA-based interindividual comparison for each call type for five individuals. All other parameters of squeaks and yap-squeaks, not included in Table 2, showed significant differences at the $p < 0.001$ level.

Table 2: Values (mean \pm SD) of high-frequency call (squeak), low-frequency call (yap) and biphonic call (yap-squeak) parameters for five dholes and results of ANOVA-based interindividual comparisons for each call type

Call parameters	Female 10	Male 11	Female 12	Male 14	Male 15	F _{4,145}
Squeak (n = 150, 30 per animal)						
g0_max, kHz	8.76 \pm 0.91	8.08 \pm 0.26	7.51 \pm 0.30	5.92 \pm 0.14	8.13 \pm 0.16	169**
g_dom, kHz	8.43 \pm 1.01	7.87 \pm 0.26	7.13 \pm 0.28	5.72 \pm 0.13	7.88 \pm 0.13	139**
g_dur_inc, ms	52 \pm 36	80 \pm 32	99 \pm 40	78 \pm 38	38 \pm 34	13**
g_dur_dec, ms	50 \pm 44	35 \pm 40	21 \pm 30	44 \pm 34	69 \pm 31	7**
Yap (n = 150, 30 per animal)						
f0_max, kHz	0.94 \pm 0.11	1.00 \pm 0.15	1.00 \pm 0.18	1.01 \pm 0.12	0.95 \pm 0.10	1.6; p = 0.16
f_dom, kHz	1.62 \pm 0.77	1.54 \pm 0.80	1.67 \pm 0.94	1.72 \pm 0.76	2.07 \pm 0.84	1.8; p = 0.13
f_dur_inc, ms	38 \pm 18	40 \pm 21	34 \pm 16	42 \pm 18	37 \pm 14	1.0; p = 0.42
f_dur_dec, ms	33 \pm 13	29 \pm 19	32 \pm 19	21 \pm 13	20 \pm 13	4.1*
Yap-squeak (n = 150, 30 per animal)						
g0_max, kHz	9.27 \pm 0.49	7.91 \pm 0.14	7.27 \pm 0.16	5.98 \pm 0.29	8.25 \pm 0.25	511**
g_dom, kHz	8.62 \pm 0.88	7.74 \pm 0.14	6.84 \pm 1.20	5.76 \pm 0.19	7.76 \pm 0.10	78**
g_dur_inc, ms	29 \pm 27	41 \pm 39	71 \pm 40	55 \pm 35	26 \pm 37	8**
g_dur_dec, ms	83 \pm 38	75 \pm 44	47 \pm 36	63 \pm 41	88 \pm 47	5*
f0_max, kHz	1.02 \pm 0.09	1.22 \pm 0.10	1.13 \pm 0.14	1.16 \pm 0.09	1.27 \pm 0.09	27**
f_dom, kHz	1.57 \pm 0.62	1.90 \pm 0.61	1.67 \pm 0.66	2.08 \pm 0.54	1.50 \pm 0.52	5**
f_dur_inc, ms	29 \pm 21	37 \pm 24	8 \pm 14	17 \pm 14	48 \pm 14	24**
f_dur_dec, ms	52 \pm 18	49 \pm 12	42 \pm 16	39 \pm 14	29 \pm 11	13**

*p < 0.01; **p < 0.001.

For yaps, significant individual differences did occur also in f0_end (p < 0.001), f0_min (p < 0.05) and in f_k_max (p < 0.05).

Three discriminant analyses were performed, based on: (1) 10 parameters of the squeak; (2) 10 parameters of the yap; and (3) 10 parameters of the yap-squeak, selected using a stepwise discriminant analysis.

For the squeak, 80.7% correct assignment was achieved (Table 3, Fig. 2a), significantly more than the random value of 30.7% ($\chi^2 = 73.96$, d.f. = 1, p < 0.001), being calculated by using the randomization procedure. The first discriminant function correlated basically with fundamental frequency parameters, primarily with g0_max and g0_end, as well as with g_peak, and explained 85.72% of the variance. Contribution of other parameters was small. The second discriminant function described 8.88% of the variance and correlated mainly with g_dur_inc, g_k_max and g0_ini (Table 4). Cross-validation analysis showed 84.0% correct assignment for the training call set (n = 75, 15 calls per individual), with 66.7–100% for particular individuals. Correct assignment for the test call set (n = 75, other 15 calls per individual) did not differ significantly from the training percentage of assignment, and showed 72.0%, varying from 33.3% to 100% among individuals ($\chi^2 = 2.49$, d.f. = 1, p = 0.11).

For the yap, the discriminant analysis showed only 44.7% correct assignment to individual (Table 3,

Table 3: Assignment of dhole calls to a predicted individual, based on discriminant analysis of the squeak, yap and yap-squeak call parameter values

Actual group	Predicted group membership					Total	Correctly classified (%)
	10	11	12	14	15		
Squeak							
Female 10	17	4	5	0	4	30	56.7
Male 11	0	22	3	0	5	30	73.3
Female 12	0	1	25	1	3	30	83.3
Male 14	0	0	0	30	0	30	100
Male 15	0	1	2	0	27	30	90.0
Total	17	28	35	31	39	150	80.7
Yap							
Female 10	13	8	2	4	3	30	43.3
Male 11	12	5	6	6	1	30	16.7
Female 12	4	4	16	2	4	30	53.3
Male 14	1	5	3	11	10	30	36.7
Male 15	3	2	1	2	22	30	73.3
Total	33	24	28	25	40	150	44.7
Yap-squeak							
Female 10	30	0	0	0	0	30	100
Male 11	0	28	1	0	1	30	93.3
Female 12	0	2	28	0	0	30	93.3
Male 14	0	0	0	30	0	30	100
Male 15	0	1	0	0	29	30	96.7
Total	30	31	29	30	30	150	96.7

Fig. 2b). This value of correct assignment did not differ significantly from the random value of 33.3% ($\chi^2 = 3.59$, d.f. = 1, p = 0.06). Both frequency and

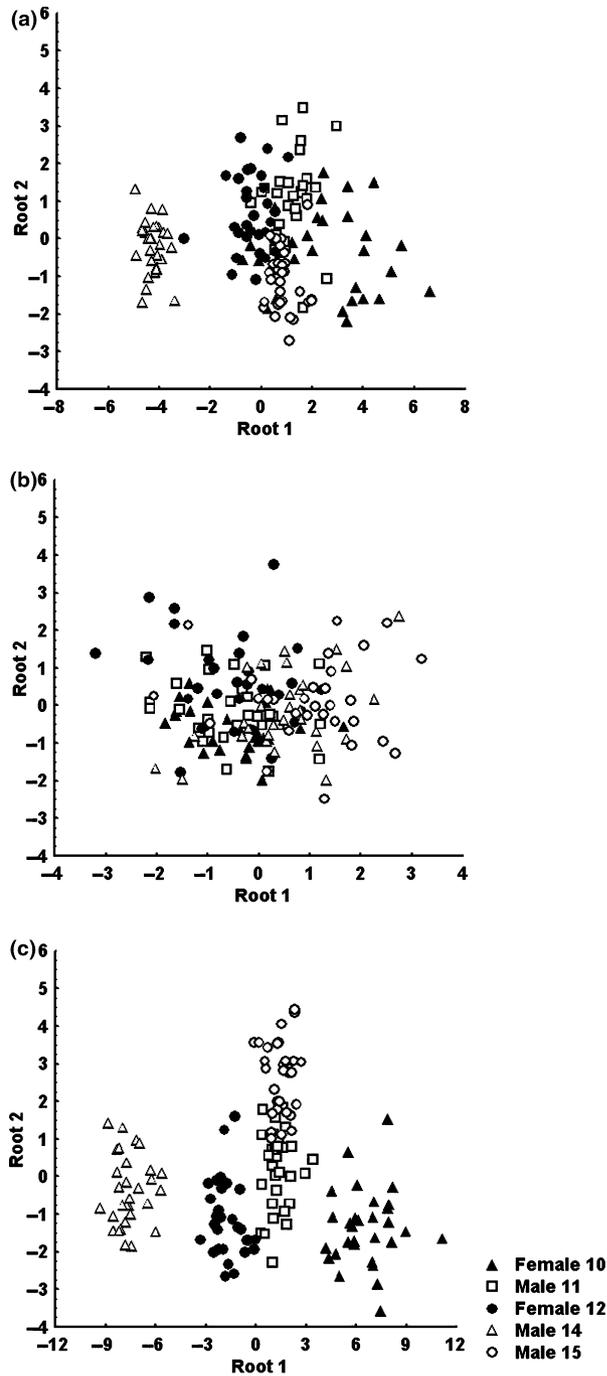


Fig. 2: Scatterplots showing separation produced by the first two discriminant functions of three call types for five dholes: (a) based on parameters of the high-frequency call or squeak; (b) based on parameters of the low-frequency call or yap; (c) based on parameters of the biphonic call or yap-squeak

temporal parameters contributed to discrimination. The first discriminant function was related primarily to parameters *f0_end*, *f_dur_dec* and *f_k_max*, that

Table 4: Values of correlation between squeak call parameters and the two first discriminant functions; eigenvalues and percent variance, described by each function

Parameters	Root 1	Root 2
<i>g0_ini</i>	0.503	-0.593
<i>g0_end</i>	0.730	0.074
<i>g0_max</i>	0.915	-0.158
<i>g0_min</i>	0.511	-0.528
<i>g_peak</i>	0.827	-0.169
<i>g_bandw</i>	0.142	0.215
<i>g_extrem</i>	0.062	-0.246
<i>g_dur_inc</i>	-0.107	0.680
<i>g_dur_dec</i>	0.036	-0.537
<i>g_k_max</i>	-0.059	0.672
Eigenvalue	5.56	0.58
Percent variance	85.72%	8.88%

Table 5: Values of correlation between yap call parameters and the two first discriminant functions; eigenvalues and percent variance, described by each function

Parameters	Root 1	Root 2
<i>f0_ini</i>	-0.112	-0.007
<i>f0_end</i>	0.559	-0.235
<i>f0_max</i>	-0.100	0.205
<i>f0_min</i>	0.318	-0.294
<i>f_peak</i>	0.301	0.141
<i>f_bandw</i>	-0.131	0.444
<i>f_extrem</i>	0.229	-0.202
<i>f_dur_inc</i>	0.065	-0.261
<i>f_dur_dec</i>	-0.484	-0.037
<i>f_k_max</i>	0.423	-0.043
Eigenvalue	0.42	0.17
Percent variance	61.23%	24.24%

described 61.23% of the variance only. The second discriminant function was founded on *f_bandw*, *f0_min* and *f_dur_inc* and described 24.24% of the variance (Table 5). Cross-validation analysis showed 53.3% correct assignment for the training call set (*n* = 75, 15 calls per individual), with 40.0–60.0% for particular individuals. Correct assignment for the test call set (*n* = 75, other 15 calls per individual) was only 34.7% (26.7–46.7% between individuals), significantly lower than the results for the training set ($\chi^2 = 4.57$, d.f. = 1, *p* < 0.05). So, the yap parameters showed low ability to discriminate between individuals.

For the yap-squeak, the stepwise discriminant analysis selected three parameters of the high-frequency component and seven parameters of the low-frequency component that contributed mostly to discrimination of calls to individuals (Table 6).

Table 6: Values of correlation between 10 yap–squeak call parameters, selected with the stepwise discriminant procedure, and the two first discriminant functions; eigenvalues and percent variance, described by each function

Parameters	Root 1	Root 2
g0_end	0.850	-0.077
g0_max	0.792	0.170
g_bandw	0.075	-0.117
f0_ini	-0.086	-0.040
f0_max	-0.057	0.509
f_bandw	0.032	-0.031
f_extrem	0.092	0.059
f_dur_inc	0.087	0.437
f_dur_dec	0.051	-0.321
f_k_max	0.058	0.542
Eigenvalue	22.27	2.12
Percent variance	87.27%	8.30%

With these 10 parameters, standard discriminant analysis provided 96.7% correct assignment (Table 3, Fig. 2c). This value is significantly higher than the random value of 32.0% ($\chi^2 = 133.88$, d.f. = 1, $p < 0.001$). In this case, the first discriminant function described 87.27% of the variance and was correlated only with fundamental frequency parameters of high-frequency component (g0_end and g0_max). The second discriminant function was based on the low-frequency component parameters, both temporal (f_k_max and f_dur_inc), and frequency (f0_max), and described as little as 8.3% of the variance (Table 6). Thus, the high-frequency component parameters contributed more to individual discrimination of biphonic calls, and the same parameters (g0_end and g0_max) that contributed mainly to discrimination of yap–squeaks were among the three that contributed mainly to discrimination of squeaks (Tables 4 and 6). Therefore, the first discriminant function of the biphonic call relied on the high-frequency parameters, whereas the second one on the low-frequency parameters (Table 6, Fig. 2), that resulted in a small increase in the percentage of correct assignment to individuals. Cross-validation analysis showed 94.7% correct assignment for the training call set ($n = 75$, 15 calls per individual), with 86.7–100% for particular individuals. Correct assignment for the test call set ($n = 75$, other 15 calls per individual) did not differ from the training percentage of assignment, and also showed 94.7%, varying from 86.7% to 100% among individuals.

Finally, a comparison of discrimination percentages to individuals between the yap, squeak and yap–squeak showed that the discriminability was

significantly higher for the squeak than for the yap ($\chi^2 = 40.02$, d.f. = 1, $p < 0.001$) and for the yap–squeak than for the yap ($\chi^2 = 95.84$, d.f. = 1, $p < 0.001$) and for the squeak ($\chi^2 = 17.55$, d.f. = 1, $p < 0.001$).

Discussion

The presented data support the hypothesis that the biphonic calls, representing a combination of the high- and the low-frequency components, enhances the potential for individual discrimination in the dhole. However, the high-frequency squeak had substantially higher potential for individual discrimination than the low-frequency yap. The yap did not provide cues to individuality at all, showing a discrimination ability that did not differ significantly from the random values.

For many canids, discriminant analysis-based research has suggested a potential for individual recognition by long-distance calls. Such data were reported for howling of timber wolves (Tooze et al. 1990), for bark series of arctic foxes *Alopex lagopus* (Frommolt et al. 1997, 2003) and swift foxes *Vulpes velox* (Darden et al. 2003), for hoo-calls of African wild dogs (Hartwig 2005) and corresponding to hoo-call vocalization of dholes (Durbin 1998). Probably, the cues to individuality in distant calls of canids compensate for the absence of visual and olfactory stimuli that provide cues to individuality in close proximity.

Our study showed the presence of individual cues in short-distance, low-intensity calls in the dhole. For short-distance calls, the necessity of individual cues is questionable, because the roles of visual and olfactory channels are considered as much more meaningful for short-distance communication. However, Owren & Rendall (1997, 2001) showed, that for group-living primates with a complex system of subordination, individual cues may also be important for short-distance calls, because calls of particular individuals adopt a role of conditioned stimuli, evoking pleasant or unpleasant effects.

Consistent with this model, dholes might use individually distinctive short-distance calls to support stable social relationships within a pack. Biphonic calls are emitted in peaceful short-distance interactions (Volodin et al. 2001), and thus their production itself may evoke positive affiliative effects in pack fellows and result in very low intrapack aggression, a characteristic for this species (Johnsingh 1982; Ludwig & Ludwig 2000). Such an effect of vocalizations has been shown for complex primate

societies: if high-ranking animals emit grooming-associated affiliative calls when approaching subordinates, positive interactions occur more often, than if they are silent (Bauers & de Waal 1991; Cheney et al. 1995).

Although the effect-conditioning model outlines the role of vocal tract formants as cues to individual identity in short-distance primate calls, such as low-frequency non-biphonic grunt and 'coo' calls (Owren & Rendall 1997, 2001; Rendall et al. 1998), in the dhole the individual cues are based on a very complex call structure, resulting from appearance of a second fundamental frequency. With the absence of formant cues in dhole calls (Volodin et al. 2001), just the use of a second fundamental frequency allows dholes to enhance strongly the potential for individual recognition of short-distance calls. The key role of two frequencies in a call spectrum for both parent-chick and mate-mate recognition was also demonstrated for two penguin species (Aubin et al. 2000; Lengagne et al. 2001; Aubin & Jouventin 2002).

Furthermore, exploitation of calls with two frequencies, lying far apart from each other, may provide additional advantages: cues to orientation and direction of a movement of pack members emitting these calls. These proposals come from physical frameworks, suggesting that high frequencies, propagated in the environment, attenuate much more strongly, than low frequencies (Wiley & Richards 1978; Roberts et al. 1980; Owings & Morton 1998; Naguib & Wiley 2001). For canids, this effect was experimentally confirmed for the domestic dog (Frommolt & Gebler 2004).

Our recent data showed that both the biphonic yap-squeaks and non-biphonic yaps provide information about orientation of a caller to a listener: when dholes called toward a microphone, the proportion of energy in the higher part of the call spectrum (above 5 kHz) was significantly higher than when calls were emitted in an orientation away from a microphone (Volodin et al. 2005a). The data, available for two dolphin species, also showed equivocal relations between directionality and presence of two frequencies in call spectra. For the killer whale, only using biphonic calls provides reliable information about orientation of a caller to a listener, whereas calls consisting exclusively of the low-frequency component, do not provide such information (Miller 2002). On the other hand, for the Hawaiian spinner dolphin *Stenella longirostris*, non-biphonic calls provided information about direction of a caller's movement just as a consequence of the difference in directionality and propagation

ability of fundamental frequency and higher harmonics (Lammers & Au 2003).

Taken together, the available data show that a wide frequency spectrum with widely spaced frequency bands alone is sufficient for coding orientation of a caller to a listener. However, as the amplitude of higher-ordered harmonics decreases about 6–12 dB per octave (Titze 1994; Owren & Bernacki 1998), an addition of a second higher fundamental frequency, lying apart from the first one, makes the biphonic call structure especially suitable for coding orientation. Indeed, both Miller's data on the killer whale and our data on the dhole showed a tendency for better performance of biphonic calls in coding orientation of a caller to a listener in comparison with monophonic calls (Miller 2002; Volodin et al. 2005a).

Therefore, in the dhole, the high-frequency squeak, occurring singly, possesses the ability to discriminate between individuals, although less well than the biphonic yap-squeak, but it could not provide cues to the orientation of a caller, because the high-frequency narrow-band calls are the most difficult to locate (Marler 1955; Klump & Shalter 1984). On the other hand, the low-frequency yap, occurring singly, possesses the ability to encode the orientation of a caller to a listener that is comparable with, but not as good as, the biphonic yap-squeak, and at the same time shows poor discrimination ability. Joined together into a biphonic call, they perform better in both respects.

The combination of enhanced potential to code individuality with enhanced potential to code orientation of a caller to a listener makes biphonic calls especially appropriate for delicate communication in a pack with complex subordination between animals, living in close vicinity. This conclusion is in accordance with our previous data, suggesting a very high occurrence of biphonic calls in the dhole (Volodin & Volodina 2002). Moreover, for the African wild dog, a second extremely social canid species, a very high level of occurrence of biphonic contact calls was reported (Wilden 1997; Wilden et al. 1998). Only two canid species – the dhole and the African wild dog – show such high percentages of production of biphonic calls, although many other canids are able to produce two fundamentals simultaneously (Nicol'skii & Frommolt 1989; Solomon et al. 1995; Riede et al. 2000; Volodin et al. 2005b). It is probable, that in conditions of dense vegetation and large social groups, under which these species live, the acoustical channel, providing information about individuality and spacing of animals, becomes

preferable for prompt communication even over short distances, releasing the visual channel from communicative load (e.g. Morton & Shalter 1977; Lamprecht et al. 1985; Manser 1999). However, further research is necessary to reveal the more clear communicative meaning of biphonic calls, using playbacks and studying auditory perception.

Unfortunately, to date, there are no data concerning the possible mechanism of production of the high-frequency squeak-like calls in canids. Our observations of dholes and domestic dogs (E. Volodina, I. Volodin, unpubl. data) showed that during emission of the squeak the mouth is closed, with sound passing exclusively through the nose. As soon as the animal begins adding a low-frequency yap, it opens its mouth. Thus the high frequency is emitted into the environment through the nose, whereas the lower is emitted through the mouth. These observations are in accordance with X-ray video data on vocalizing domestic dogs, showing that high-frequency whines were produced nasally, whereas low-frequency barks were produced with open mouth (Fitch 2000).

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