

Sign and strength of emotional arousal: vocal correlates of positive and negative attitudes to humans in silver foxes (*Vulpes vulpes*)

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Summary

The hypothesis of similarity in trends of acoustic characteristics regardless of the sign of emotional arousal, positive or negative, has been advanced based on human vocalizations. For non-human mammals, testing is complicated because the same stimulus cannot evoke opposite (positive and negative) internal states, to trigger the respective vocalizations. To resolve this concern, we designed an experimental procedure using Tame and Aggressive strains of silver foxes, with genetically predetermined positive or negative emotional responses to humans respectively. We analyzed features of vocalizations produced by callers at different fox-human distances, assuming changes in vocal responses reflect the shifts of human-related positive arousal in Tame foxes and human-related negative arousal in Aggressive foxes. Both strains showed similar trends for changes in calling rate and proportion of time spent vocalizing toward higher levels in response to greater emotional arousal, positive in Tame foxes and negative in Aggressive foxes. At the same time, strains showed distinctive trends for the proportions of different call types and maximum amplitude frequency. We infer that the variables with similar trends reflect the strength of emotional arousal, regardless of triggering internal states, whereas variables with distinctive trends are specifically related to the sign of emotion in silver fox.

Keywords: vocalization, human-approach test, emotional arousal, acoustic communication, Canidae.

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Introduction

Answering the questions surrounding the relationship between vocal structure and emotional arousal is of utmost importance. From a theoretical perspective vocal expressions of emotion are a common feature of human and non-human communication (Darwin, 1872; Bachorowski & Owren, 2003, 2008). From a practical perspective vocal correlates of emotional arousal can provide immediate and reliable indicators of welfare problems both in humans (e.g., Furlow, 1997; Protopapas & Lieberman, 1997) and in non-human animals (e.g., Weary & Fraser, 1995b; Watts & Stookey, 2000; Gogoleva et al., 2010a).

The term emotion is ambiguous because it is used differently in psychology, neuroscience and behaviour studies. More than a hundred definitions for the term emotion and the related terms (affect, motivational state, internal state, emotional quality) can be found in the literature, and some authors even avoid distinguishing between emotional and motivational terms (e.g., Jürgens, 1979). To date, there is no scientific consensus on emotions in animals, namely, what certain species feel and how these feelings can be examined. However, it is possible to judge unambiguously at least of the sign of emotion (negative to positive), and of the strength of emotional arousal (low to high), i.e., about trends of the negative and positive emotional arousal. Consistently, robust vocal correlates of two alternative internal states, aggression and fear, have been stated empirically across 28 mammalian and bird taxa (Morton, 1977).

Vocal correlates of the sign of emotion can be revealed from differences in vocal responses to comfort and to discomfort. Vocal correlates of gradations in emotional arousal can be revealed from shifts in values of acoustic parameters with an increase or decrease of positive or negative stimulation. For humans, vocal correlates of emotional arousal have been reported both for negative and positive emotions. In newborns an increase in positive or negative emotional arousal results in the same acoustic effect, namely in the increase of the fundamental frequency (Papousek, 1992; Scheiner et al., 2002), duration and amplitude (Papousek, 1992). In adults, responding vocally to verbal approval or censures, an increase of positive or negative emotional arousal shows similar shifts in acoustic characteristics towards a higher fundamental frequency of vowels (Bachorowski & Owren, 1995). Consistently, the amplitude, fundamental frequency, maximum amplitude frequency and

rate of pronouncing of speech sounds increase uniformly under fear, anger and joy, compared to at neutral emotional state of a speaker (Johnstone & Scherer, 2000; Scherer, 2003). These findings confirm the hypothesis that in humans vocal structures provide primarily cues that indicate the strength of emotional arousal, and only to a lesser extent, the sign of positive or negative emotion (Bachorowski, 1999; Bachorowski & Owren, 2003, 2008).

For non-human mammals, this hypothesis has not yet been tested, probably because of the skewed statistics towards studies of discomfort-related vocalizations (e.g., Weary et al., 1998; Watts & Stookey, 1999; Fichtel et al., 2001; Kirchhof et al., 2001; Pongrácz et al., 2006; Gogoleva et al., 2010a). Vocal correlates of positive emotional arousal are poorly understood for non-human mammals, as they produce calls mainly in response to negative triggering internal states, while they typically remain silent when experiencing either positive or neutral emotions (Jürgens, 1976a,b, 1979; Blumstein et al., 2006). When animals are vocal during emotionally positive contexts researchers focus only on the occurrence of particular call types that attend to positive emotions (Peters, 2002; Scheumann et al., 2007; Gogoleva et al., 2008, 2009).

A population of Belyaev's silver foxes, selected for many generations either for tame behaviour (Tame strain) or for enhanced aggressiveness toward humans (Aggressive strain) (Belyaev, 1979; Trut, 1999; Trut et al., 2006, 2009; Kukekova et al., 2008) provided a convenient model for testing the hypothesis of proposed common trends in the shift of acoustic characteristics independently from the kind of arousal, positive or negative. The great advantage of this model is in the precise knowledge of attitudes of foxes of each strain to approaching humans. Silver foxes from the Tame strain respond positively towards people, whereas the foxes from the Aggressive strain respond negatively towards people. Although neither Tame nor Aggressive strains of foxes are afraid of humans, human approach causes them to respond in a friendly or attacking manner (Trut, 1980, 1999; Kukekova et al., 2008; Gogoleva et al., 2009). Cross fostering, cross breeding and embryo transplantation experiments have shown that behavioural differences between Tame and Aggressive foxes are genetically determined (Trut, 1980, 2001). Distinctive to Tame and Aggressive foxes, unselected for behaviour farmed silver foxes (Unselected) normally show aggressive-fearful responses to humans and try to increase their distance from an approaching human (Pedersen, 1994; Trut, 1999; Kukekova et al., 2008; Gogoleva et al., 2010a).

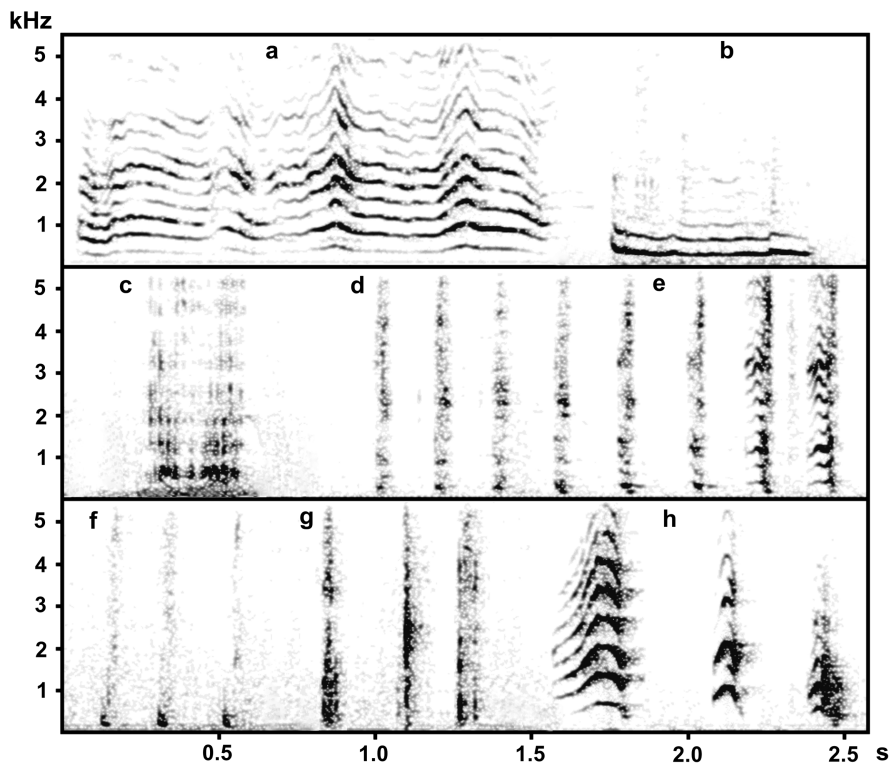


Figure 1. Spectrogram illustrating call types of silver foxes toward an approaching human: (a) whine of a Tame fox, (b) moo of an Unselected fox, (c) growl of an Unselected fox, (d) pant of a Tame fox, (e) cackle of a Tame fox, (f) snort of an Unselected fox, (g) cough of an Aggressive fox, (h) bark of an Aggressive fox.

Gogoleva et al. (2008) described eight call types produced by Tame, Aggressive and Unselected silver foxes toward people: five tonal (whine, moo, cackle, growl and bark), and three noisy (pant, snort and cough) (Figure 1). In other previous studies we examined vocal types produced by different fox strains and their hybrids toward people (Gogoleva et al., 2009) and toward conspecifics (Gogoleva et al., 2010b). In the presence of humans, Tame foxes produced cackles and pants but never coughed or snorted, while Aggressive foxes produced coughs and snorts but never cackled or panted. Wild-type Unselected foxes produced cough and snort toward humans similarly to Aggressive foxes. In the presence of conspecifics, vocal responses were similar in Tame, Aggressive and Unselected fox strains.

Percentages of particular call types attending emotionally positive and emotionally negative contexts have been estimated previously for Tame, Aggressive and Unselected foxes (Gogoleva et al., 2008, 2009, 2010b). A study of vocal expression of negative emotional arousal (low to high) toward humans in Unselected foxes (Gogoleva et al., 2010a) revealed that the overall calling rate, time spent vocalizing and shifts in values of power variables of the 'joint calls' most reflected the vocal expression of emotional intensity. Joint calls envelope all calls produced at a given test step, in order to take into account the acoustic characteristics of all calls independently of their structure, tonal or noisy. Joint calls better reflected shifts in acoustic characteristics with changes in emotional arousal of animals compared with acoustic characteristics measured within calls of different types (Gogoleva et al., 2010a).

Our general purpose here was to reveal acoustic parameters, encoding a universal increase in emotional arousal, independent from the sign of emotional quality, positive or negative. Specifically, we apply here the same experimental design and the same algorithm of acoustic analysis as in Gogoleva et al. (2010a), to Tame and Aggressive strains of silver foxes, in order to examine the features of vocalizations produced by the animals at different fox-human distances. We assume that changes in vocal responses reflect the intensity of human-related positive emotional arousal in Tame foxes and of negative emotional arousal in Aggressive foxes. Finally, we compared trends in the vocal features of the two strains to estimate whether they were uniform for the positive or negative emotional enforcement.

Materials and methods

Subjects, site and dates of work

Our subjects were 50 adult (1–3 years old) female silver foxes, kept at the experimental fur farm of the Institute of Cytology and Genetics, Novosibirsk, Russia. Two study groups included 25 Tame (selected for tameness toward humans, 45–47 generations since the start of selection) and 25 Aggressive (selected for aggressiveness toward humans; 34–36 generations since the start of selection) vixens. The foxes were kept and tested in individual outdoor cages (70 × 85 × 90 cm) with a wire mesh floor. The cages were arranged in batteries of 50 cages per row, with two rows opposite each other

and a 1.7 m wide passageway between them. The cages were covered with a slate roof with two sloping surfaces providing protection from wind, rain and hot sun. Foxes were fed twice a day (beef, meat by-products, minced chicken, cereals, vitamins and minerals). Water was available *ad libitum*. As early exposure to humans can effect future reactions of foxes to people it is forbidden to establish personal contacts with any particular fox on this experimental farm. Fox pups socialize with conspecifics when they live together with their mothers until weaning and then live together with their littermates up to separation into individual cages at the age of 3 months. After separation they remain in visual, olfactory and auditory contact with foxes from neighboring and opposing cages. This holding regime has been standardized since 1960 and is uniform for all foxes on the farm, thereby excluding the influence of new factors on the behaviour of these animals. Human-approach tests were made when foxes were in their home cages, out of breeding or pup raising seasons, from July–August 2006 and in June 2007.

Experimental procedure

The same researcher (S.S.G.), unfamiliar to the foxes, performed all human-approach tests (one per fox), with parallel acoustic recordings. Each test lasted 10 min and included five successive steps, each lasting 2 min. Transitions between the successive test steps were regulated using watches and were labeled by voice. A test started at the moment of the researcher's approach to a focal fox cage at a distance of 50 cm. At Step 1 the researcher was motionless; at Step 2 performed smooth body and hand movements left to right, maintaining a distance of 50 cm; at Step 3 the researcher shortened the human–fox distance with one step forward, and performed body and hand movements forward and back, touching the cage door with her fingers; at Step 4 the researcher enhanced the human–fox distance with one step back; and repeated Step 2; and the Step 5 repeated Step 1. Thus, the human impact on an animal increased between the Steps 1 and 3 and decreased between the Steps 3 and 5. For Tame foxes, Step 3 differed in that the researcher opened the cage door and caressed the fox for the duration of this step. This additional positive enforcement was necessary to elicit a noticeable shift of emotional arousal in Tame foxes from Step 2 to Step 3. A previous full-scale series of experiments with a separate sample of Tame foxes revealed that without caressing they do not show any shift in the level

of arousal at the central Step 3, despite the decrease in fox–human distance from Step 2 to Step 3. The shifts in the levels of emotional arousal were estimated by behaviour of foxes, by levels of their activity and by striving to approach the front door (i.e., by striving to contact with a human either friendly or aggressively).

During a test, the focal animal stared and interacted in particular with the human, evidently responding to the actions of the experimenter. In contrast, no visible responses to neighboring foxes have been observed, and behaviour of the human represented much stronger stimulus for the focal fox than behaviour of neighboring foxes.

Acoustic recording

We used a Marantz PMD-222 (D&M Professional, Kanagawa, Japan) cassette recorder with an AKG-C1000S (AKG-Acoustics, Vienna, Austria) cardioid electret condenser microphone, and Type II chrome audiocassettes EMTEC-CS II (EMTEC Consumer Media, Ludwigshafen, Germany). The system had a frequency response of 0.04–14 kHz at a tape speed of 4.75 mm/s.

The distance between the microphone and a focal fox varied from 25 to 100 cm; the orientation of an animal to the microphone was mostly frontal or lateral. If a non-focal fox called simultaneously with the focal fox, the calls of the focal fox were labeled by voice. The labeling of calls by voice is a traditional practice that is necessary in the event that more than one animal calls simultaneously. This technique allows calls of focal and other animals to be distinguished during the following analysis.

Acoustic analysis

Successive call digitizing (with each test step taken as a separate file) at 22.05 kHz sampling rate, 16 bit precision; high-pass filtration at 0.1 kHz and measurements were made with Avisoft-SASLab Pro v. 4.33 (Avisoft Bioacoustics, Berlin, Germany). Spectrograms for analysis were created using Hamming window, FFT-length 1024 points, frame 50%, and overlap 87.5%. Figure spectrograms were created with calls downsampled to 11.025 kHz, Hamming window, FFT-length 512 points, frame 50%, and overlap 87.5%.

By spectrogram, one researcher (S.S.G.) classified each call visually to one of eight types (Figure 1) according to the vocal traits described in Gogoleva et al. (2008), blindly to the fox strain and to the number of the test step

during which the calls were recorded. The sound utterances were considered as separate calls if they were separated by a silence space longer than 20 ms. In total, we examined 7885 calls from Tame foxes and 7800 calls from Aggressive foxes.

To calculate the proportion of time spent vocalizing per fox per test step, i.e., the total duration of calls within a step divided by the duration of the step in min (taken in %), we measured the duration of a given test step and the duration of each call produced during this test step with the standard marker cursor in the main window of Avisoft. The measurements were exported automatically to Excel (Microsoft, Redmond, WA, USA). To calculate the calling rate (calls/min), we divided the number of calls by the duration of the test step (in min).

In addition, we prepared 'joint calls', cutting off the silent spaces between calls of a focal fox produced within a test step (Figure 2, see Gogoleva et al., 2010a). In total, we prepared 105 joint calls for the 21 of 25 Tame foxes and 120 joint calls for the 24 of 25 Aggressive foxes, one joint call per fox per test step. Since four Tame and one Aggressive fox were silent during one of the test steps, we excluded these five individuals from analysis of joint calls. Using the automatic parameter measurement option of Avisoft, we measured the maximum amplitude frequency ('dominant frequency' or 'peak frequency') from the mean power spectrum of each joint call (Figure 2).

Statistics

All statistical analyses were carried out with Statistica v. 6.0 (StatSoft, Tulsa, OK, USA). All tests were two-tailed and differences were considered significant where $p < 0.05$. We used GLM for repeated measures with the Newman–Keuls post hoc test to compare the vocal traits between the test steps. We used GLM for repeated measures (with test step as the repeated factor and study group as the fixed factor) with the F -test as post hoc to compare the vocal traits between Tame and Aggressive strains. Since the values of acoustic parameters (calling rates, proportions of the time spent vocalizing and maximum amplitude frequencies of joint calls) did not satisfy the criteria of normality with the Kolmogorov–Smirnov test, we root-square transformed the data to introduce them into GLM. We also used a χ^2 test with Fisher exact test post hoc, to compare the proportions of calls of different types between the successive steps of the human-approach test.

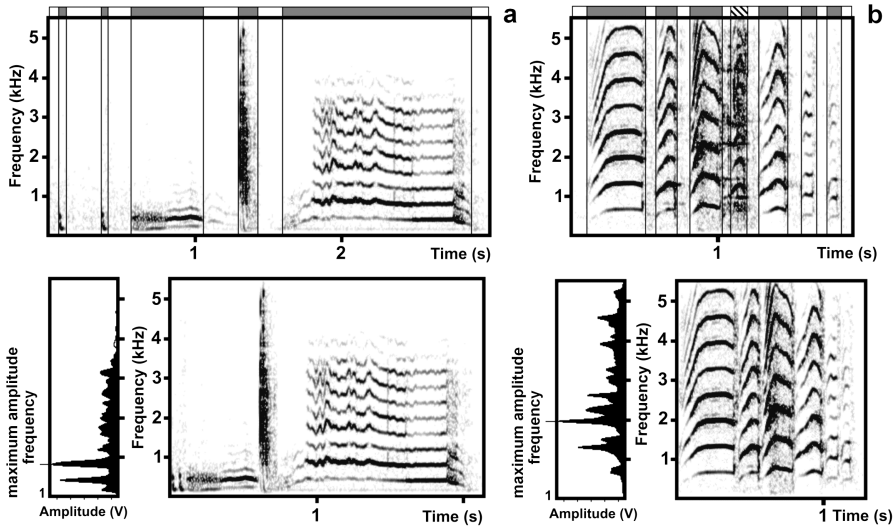


Figure 2. Procedure for preparation of a joint call and maximum amplitude frequency measure: (a) Aggressive fox and (b) Tame fox. The joint calls take into account the acoustic characteristics of all calls independent of their structure, tonal or noisy. Above: Spectrogram of an intact natural sequence of calls, produced by a focal silver fox during a human-approach test. The sequence contains tonal and noisy calls (labeled with dark bars above the spectrogram), separated with silent spaces (labeled with light bars above the spectrogram). The striped bar labels the superimposed call of a non-focal neighboring fox, produced simultaneously with the call of the focal fox. Below: Spectrogram and power spectrum of a part of the future joint call, made from the call sequence shown above. Only dark-labeled fragments are reserved, while the light-labeled and strip-labeled are cut off.

Results

Study foxes produced calls of eight types: whines, moos, growls, coughs, snorts, cackles, pants and barks (Figure 1). The numbers of Tame and Aggressive callers that produced each call type varied between the successive steps of the human-approach test (Table 1). No one call types could be found in all individuals at all the five test steps. Only 4 Tame and 1 Aggressive fox remained silent during one of the test steps.

Only whine, moo and growl were found in both strains. Tame foxes also produced cackle and pant but not cough, snort or bark. In contrast, Aggressive foxes also produced cough, snort and bark, but not cackle or pant. Growl and bark were the rarest vocalizations, found only in 1 Tame and 9 Aggressive foxes (growl) and from 1 Aggressive fox (bark). Whine was a single call type, often found in both the strains at all the test steps (Table 1). In the

Table 1. Cells show quantities of animals from the Tame or Aggressive fox strains, produced calls of certain call type at successive steps of a human-approach test, total quantities of all foxes called at the give test step, and total sums produced by all callers at the given step.

Call type	Tame foxes					Aggressive foxes				
	Step 1	Step 2	Step 3	Step 4	Step 5	Step 1	Step 2	Step 3	Step 4	Step 5
Whine	14	13	15	11	11	15	16	20	18	14
Moo	3	1	5	1	2	21	22	23	23	21
Growl	0	0	1	0	0	1	0	3	3	7
Cough	0	0	0	0	0	15	21	24	21	8
Snort	0	0	0	0	0	17	19	15	14	12
Cackle	11	13	11	8	5	0	0	0	0	0
Pant	18	25	21	22	17	0	0	0	0	0
Bark	0	0	0	0	0	0	0	0	1	0
All call types	24	25	24	25	23	25	25	25	25	24
Number of calls	1408	2146	2809	881	641	1132	1782	2810	1408	668

Tame strain, the numbers of growls, barks and moos were too small to allow inclusion of these call types into analysis, and in the Aggressive strain, the numbers of growls and barks were also too small to allow inclusion of these call types into analysis.

Tame and Aggressive strains showed distinctive trends for the proportions of different call types throughout the test (Figure 3). In Tame foxes, χ^2 tests showed that proportions of calls of different types differed significantly between Steps 1 and 2 ($\chi^2 = 221.6$, $df = 2$, $p < 0.001$), Steps 2 and 3 ($\chi^2 = 143.8$, $df = 2$, $p < 0.001$), Steps 3 and 4 ($\chi^2 = 90.4$, $df = 2$, $p < 0.001$) but not between Steps 4 and 5 ($\chi^2 = 0.3$, $df = 2$, $p > 0.05$). In Aggressive foxes, χ^2 tests showed that proportions of calls of different types differed significantly between Steps 1 and 2 ($\chi^2 = 136.9$, $df = 3$, $p < 0.001$), Steps 2 and 3 ($\chi^2 = 106.0$, $df = 3$, $p < 0.001$), Steps 3 and 4 ($\chi^2 = 247.6$, $df = 3$, $p < 0.001$) and Steps 4 and 5 ($\chi^2 = 116.8$, $df = 3$, $p < 0.001$).

In Tame foxes, a Fisher exact post hoc test showed that the proportion of pants increased significantly between Steps 1 and 2, and decreased significantly between Steps 3 and 4. The proportion of cackles decreased significantly between Steps 1 and 2 and between Steps 2 and 3, and increased significantly between Steps 3 and 4. The proportion of whines decreased significantly between Steps 1 and 2 and increased significantly between Steps

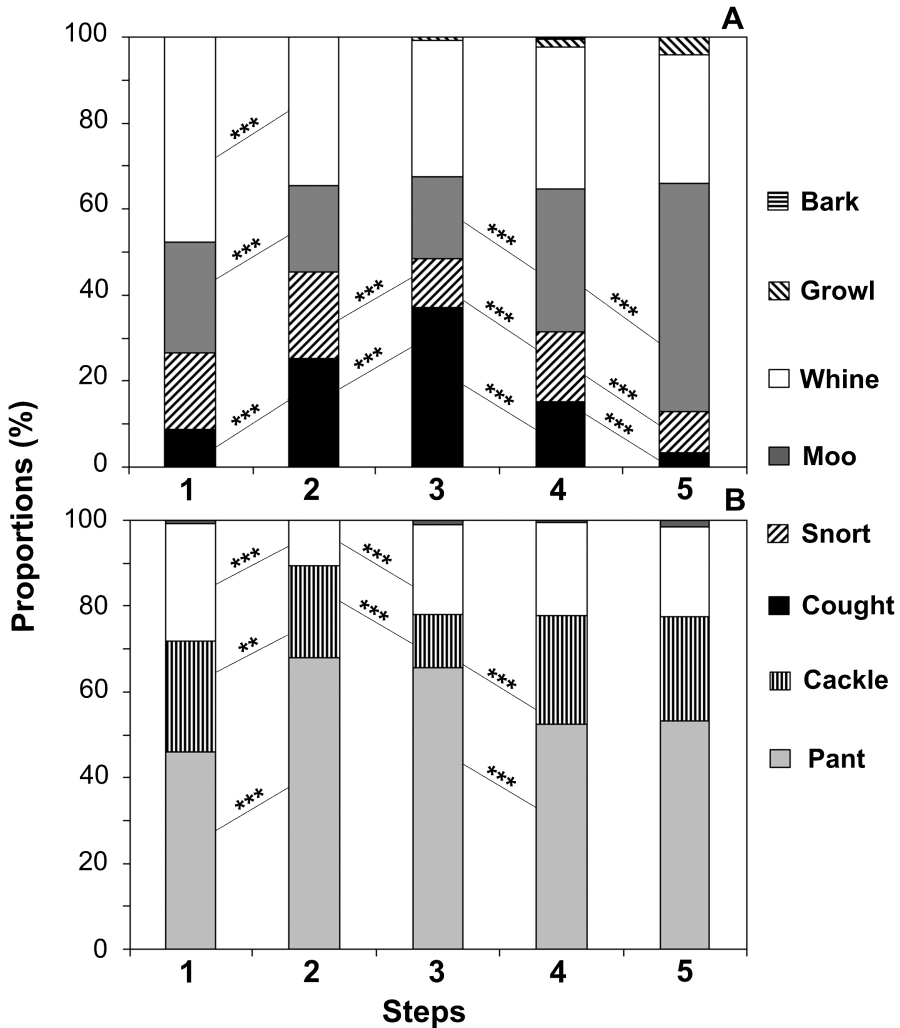


Figure 3. Proportions of calls of different types for five successive steps of a human-approach test applied to Tame (A) and Aggressive (B) silver foxes and comparison between adjacent steps with Fisher exact test: ** $p < 0.01$; *** $p < 0.001$.

2 and 3 (Figure 3A). In Aggressive foxes, Fisher exact post hoc test showed that the proportion of coughs increased significantly between Steps 1 and 2 and between Steps 2 and 3, and decreased significantly between Steps 3 and 4 and between Steps 4 and 5. The proportion of snorts decreased significantly between Steps 2 and 3, increased significantly between Steps 3 and

Table 2. GLM for repeated measures statistics (with test step as repeated factor and study group as fixed factor) for study group effect and for combined test step and study group effect on silver fox acoustic parameter values.

Acoustic parameter	Group effect	Step and Group effect
Calling rate	$F_{1,48} = 1.89, p = 0.18$	$F_{4,192} = 1.96, p = 0.10$
Proportion of time spent vocalizing	$F_{1,48} = 12.18, p = 0.001^*$	$F_{4,192} = 1.85, p = 0.12$
Maximum amplitude frequency of joint calls	$F_{1,43} = 0.55, p = 0.46$	$F_{4,172} = 3.70, p < 0.01^*$

* Significant effect.

4 and then decreased significantly between Steps 4 and 5. The proportion of moos decreased significantly between Steps 1 and 2 and increased significantly between Steps 3 and 4 and between Steps 4 and 5. The proportion of whines decreased significantly between Steps 1 and 2 (Figure 3B).

The Tame and Aggressive fox strains showed similar degrees of calling rates and their trends of changes between steps throughout the test (Table 2, Figure 4). GLM for repeated measures revealed a significant influence of the test step on the calling rate in Tame foxes ($F_{4,96} = 9.20, p < 0.001$) and in Aggressive foxes ($F_{4,96} = 30.73, p < 0.001$). For the Tame strain calling rate, the Newman–Keuls post hoc test showed a significant increase between Steps 1 and 3 ($p < 0.05$), and a significant decrease between Steps 3 and 5 ($p < 0.001$), and between Steps 3 and 4 ($p < 0.001$) (Figure 4). For the Aggressive strain calling rate, the Newman–Keuls post hoc tests showed a significant increase between Steps 1 and 3 ($p < 0.001$), between Steps 1 and 2 ($p < 0.01$) and between Steps 2 and 3 ($p < 0.001$), and a significant decrease between Steps 3 and 5 ($p < 0.001$), between Steps 3 and 4 ($p < 0.001$), and between Steps 4 and 5 ($p < 0.001$) (Figure 4).

A trend in the proportion of time spent vocalizing matched those of the calling rate, increasing between Steps 1 and 3 and decreasing between Steps 3 and 5 in both Tame and Aggressive strains (Figure 5). GLM for repeated measures revealed a significant influence of the test step on the proportion of time spent vocalizing in Tame foxes ($F_{4,96} = 7.65, p < 0.001$) and in Aggressive foxes ($F_{4,96} = 10.12, p < 0.001$). For Tame foxes, the Newman–Keuls post hoc test showed a significant increase in time spent vocalizing between Steps 2 and 3 ($p < 0.05$), and a significant decrease in the values of this variable between Steps 3 and 4 ($p < 0.001$) and between

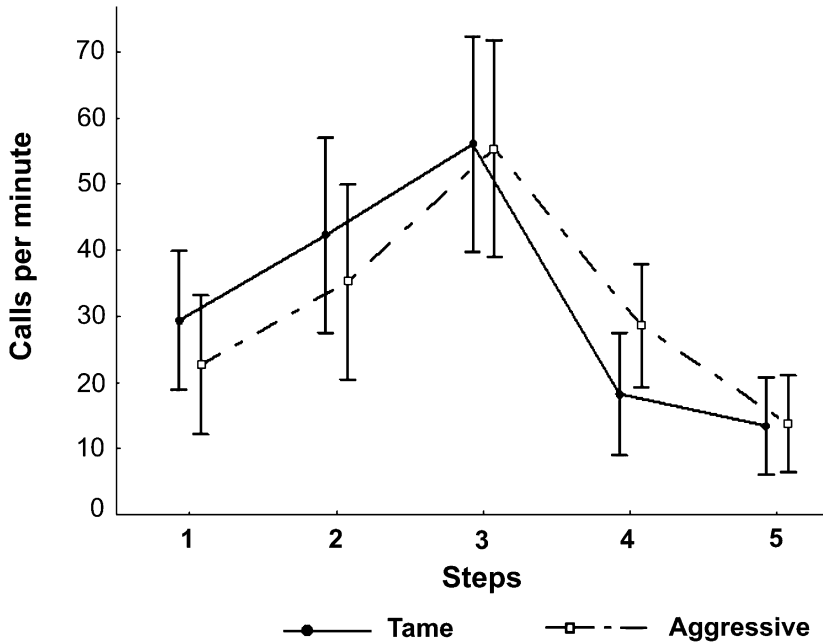


Figure 4. The values of the calling rate occurring at five successive steps of a human-approach test, applied to silver foxes of Tame (solid line) and Aggressive (dashed line) strains. Central points show means, whiskers show 0.95 confidence intervals.

Steps 3 and 5 ($p < 0.001$) (Figure 5). For Aggressive foxes, the Newman-Keuls post hoc test showed a significant increase in time spent vocalizing between Steps 1 and 3 ($p < 0.001$) and between Steps 2 and 3 ($p < 0.01$), and a significant decrease between Steps 3 and 5 ($p < 0.001$), between Steps 3 and 4 ($p < 0.01$) and between Steps 4 and 5 ($p < 0.01$) (Figure 5).

Step-by-step trends of change in the proportion of time spent vocalizing did not differ between Tame and Aggressive foxes (Table 2). However, the values of time spent vocalizing were significantly higher in the Aggressive strain (Table 2, Figure 5). Post hoc F -test showed significant differences between Tame and Aggressive foxes in the proportion of time spent vocalizing at Step 2 ($F_{1,48} = 9.78$, $p < 0.01$), Step 3 ($F_{1,48} = 8.37$, $p < 0.01$), Step 4 ($F_{1,48} = 16.58$, $p < 0.001$) and Step 5 ($F_{1,48} = 9.13$, $p < 0.01$) (Figure 5).

For the maximum amplitude frequency of joint calls, GLM for repeated measures revealed a significant influence of test step for Aggressive foxes ($F_{4,92} = 6.96$, $p < 0.001$), but not for Tame foxes ($F_{4,80} = 1.42$, $p = 0.23$) (Figure 6). For Aggressive foxes, the Newman-Keuls post hoc test showed

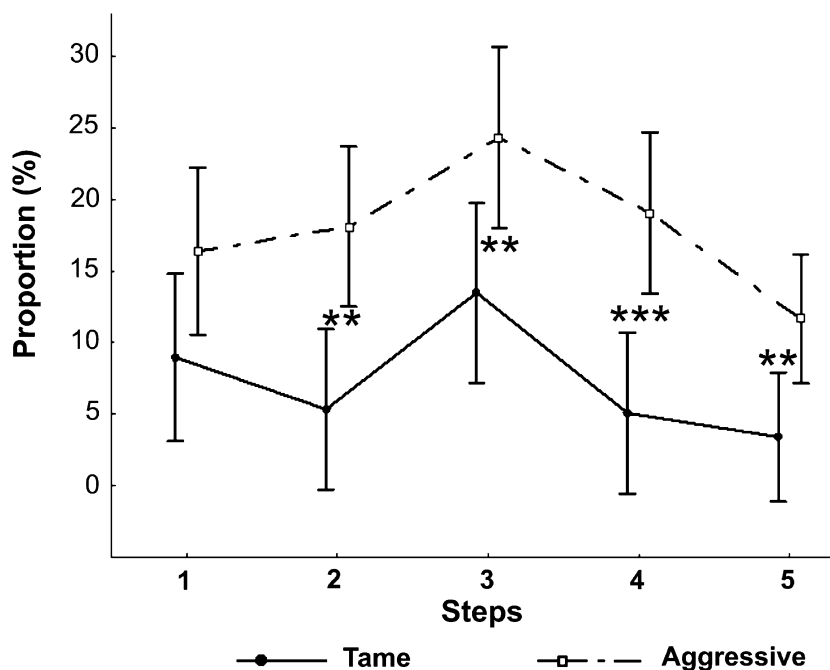


Figure 5. The values for the proportion of time spent vocalizing for five successive steps of a human-approach test, applied to Tame (solid line) and Aggressive (dashed line) strains. Post hoc F -test between Tame and Aggressive foxes: ** $p < 0.01$, *** $p < 0.001$. Central points show means, whiskers show 0.95 confidence intervals.

a significant increase in the maximum amplitude frequency between Steps 1 and 2 ($p < 0.05$) and between the Steps 1 and 3 ($p < 0.01$), and a significant decrease between Steps 3 and 5 ($p < 0.001$) and between Steps 4 and 5 ($p < 0.05$) (Figure 6).

GLM for repeated measures showed significant differences between Tame and Aggressive strains for step-by-step trends in the maximum amplitude frequency of joint calls (Table 2, Figure 6). In Tame foxes, the values of the maximum amplitude frequency of joint calls decreased steadily between Step 1 and Step 5, whereas in Aggressive foxes, they showed an increase up to Step 3 followed by a decrease. A post hoc F -test showed significant differences between Tame and Aggressive foxes in the maximum amplitude frequency of joint calls at Step 1 ($F_{1,43} = 7.15$, $p < 0.05$) and Step 5 ($F_{1,43} = 6.90$, $p < 0.05$) (Figure 6).

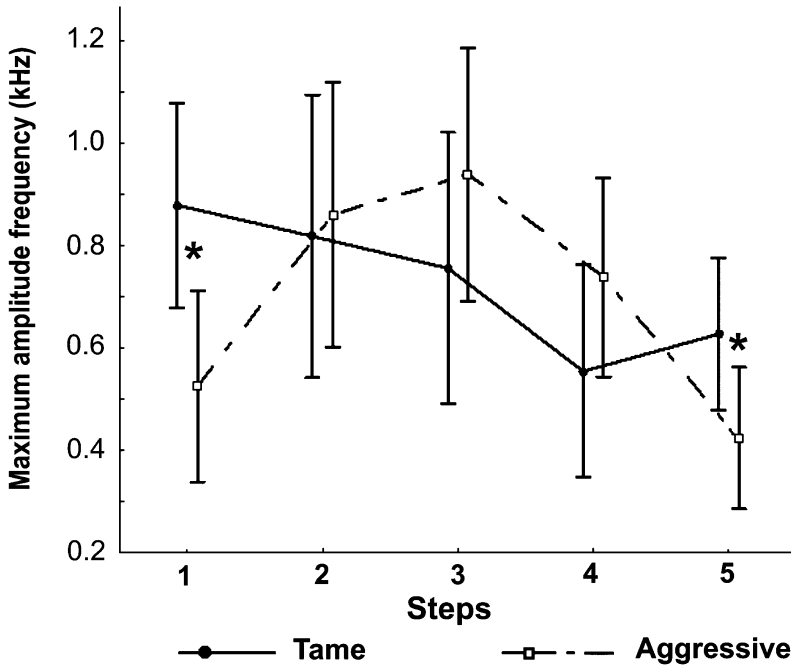


Figure 6. The values of the maximum amplitude frequency of joint calls for five successive steps of a human-approach test, applied to Tame (solid line) and Aggressive (dashed line) strain. Post hoc *F*-test between Tame and Aggressive foxes: * $p < 0.05$. Central points show means, whiskers show 0.95 confidence intervals.

Discussion

Both Tame and Aggressive silver foxes adjusted their vocal responses accordingly to changes in human-fox distance at successive steps of the human-approach test. Trends for the calling rate and for the proportion of time spent vocalizing were the same for Tame and Aggressive strains. Thus, these two acoustic variables reflected the strength of emotional arousal irrelevant of the sign of emotion, positive or negative, and could be considered as universal indicators of emotional arousal in silver fox. At the same time, trends for the maximum amplitude frequency of joint calls and for the proportions of different call types differed strongly between the strains, suggesting that they have a specific relationship to the sign of emotion.

Results of this study agree with previous studies that found the same two allied parameters, calling rate and time spent vocalizing, as indicative of the level of emotional arousal (reviews in Gould, 1983; Volodin et al., 2009).

The time spent vocalizing and the calling rate are related parameters, as the increase in time spent vocalizing can be achieved either by accounting for a greater calling rate or by greater durations of calls (Volodin et al., 2009). In red fox pups, the calling rate is reported as a critical feature of a mother's response (Tembrock, 1958). In Unselected silver foxes, the calling rate increased with increased arousal in response to human approach (Gogoleva et al., 2010a). In baboons (*Papio hamadrayas*), the calling rates of grunts were significantly higher in high-arousal compared to low-arousal conditions (Rendall, 2003). In the domestic sow (*Sus scrofa*), higher calling rates have been observed in hungry piglets compared to those fed (Weary & Fraser, 1995a; Weary et al., 1997) and under real castration compared to sham castration (Weary et al., 1998). Also, increased calling rates were found in ground-dwelling rodents under increasing threat during a predator approach (Blumstein & Armitage, 1997; Warkentin et al., 2001), and increased rates of barks were found in domestic dogs (*Canis familiaris*) in the context of 'mobbing a stranger' compared to the contexts 'left tethered' or 'soliciting for a toy' (Pongrácz et al., 2005). In tree shrews (*Tupaia belangeri*) at high-arousal, the syllables in chatter calls were longer and followed with shorter inter-syllable intervals compared to the state of low-arousal (Schehka & Zimmermann, 2009). Increased call durations were found in meerkats (*Suricata suricatta*) under increasing threat during a predator approach (Manser, 2001). In Unselected silver foxes, the time spent vocalizing increased with increasing arousal in response to human approach (Gogoleva et al., 2010a). In contrast, call durations did not differ between castrated and sham-castrated piglets (Weary et al., 1998).

Throughout the test, patterns in the maximum amplitude frequency of joint calls differed between strains, with the Tame strain starting high and descending across steps and the Aggressive strain showing an increasing and then decreasing pattern. Given these differences in trends, the maximum amplitude frequency of joint calls is indicative of sign of emotion (positive vs negative) rather than of emotional arousal. These data seem inconsistent compared to the data on Unselected foxes (Gogoleva et al., 2010a) and on other mammals. The maximum amplitude frequency and other power parameters can be applied to calls of any structure, both tonal and noisy (Owren & Bachorowski, 2007; Gogoleva et al., 2010a). The shifts of power parameter values towards higher frequencies with increased arousal have been reported for common marmosets, *Callithrix jacchus* (Schrader & Todt,

1993), Barbary macaque (*Macaca sylvanus*: Fischer et al., 1995), tree shrews (Schehka & Zimmermann, 2009), domestic sows (Weary & Fraser, 1995a; Weary et al., 1998), squirrel monkeys (*Saimiri sciureus*: Fichtel et al., 2001), domestic dogs (Pongrácz et al., 2005, 2006) and for humans (Ruiz et al., 1996). The maximum amplitude frequency appears, therefore, to be a universal parameter encoding emotional arousal in mammals (Volodin et al., 2009).

Although our data appear to be inconsistent with those reported in the studies mentioned above the inconsistency is in fact only a result of differences in methodical design, stemming from our analysis of joint calls. While the proportion of whines was similar in both fox strains, pants and cackles were used exclusively by Tame foxes, whilst moo, coughs and snorts exclusively by Aggressive foxes. Pant, cackle and cough are higher in frequency, while moo and snorts are lower in frequency (Gogoleva et al., 2008), such the proportions of these call types affected the maximum amplitude frequency of joint calls. Thus, it appears that the proportion of different call types was the most reliable acoustic indicator of quality of emotion in silver fox. In this study foxes produced calls of eight types: whines, moos, growls, coughs, snorts, cackles, pants and barks (Figure 1). Throughout the test, each fox strain produced three shared call types and two (Tame) or three (Aggressive) strain-specific call types, without adding new call types and not excluding those used previously. Aggressive foxes specifically used cough and snort, while Tame foxes used cackle and pant. The data concerning call sets produced by each of the strains are consistent with the data of previous studies, despite differences in testing procedures (Gogoleva et al., 2008, 2009). This suggests that vocal types of silver foxes in response to humans are pre-determined as a result of strict directional selection for tame or aggressive behaviour toward humans.

Similar tests, conducted with Unselected silver foxes revealed an increase in discomfort-related emotional arousal with shorter fox–human distance and the decrease of arousal with larger fox–human distance (Gogoleva et al., 2010a). Distinctive from Tame and Aggressive foxes, Unselected silver foxes are typically afraid of humans and try to increase their distance from an approaching human (e.g., Pedersen, 1994; Trut, 1999; Kukekova et al., 2008). In all three study groups, Unselected (Gogoleva et al., 2010a) Tame and Aggressive (this study) positive feedback was found between the degree

of human enforcement and the values of the proportion of time spent vocalizing and of calling rate. The values of both these parameters achieved a maximum at the point of maximum emotional arousal of the study foxes (at Step 3 of the human-approach test). Therefore, trends of these two acoustic parameters were the same in all three fox strains, despite differences in internal states experienced by the foxes.

The test procedure was the same for Aggressive and Unselected foxes, but somewhat intensified for Tame foxes. For Tame foxes, Step 3 required the researcher to open the cage door and caress the fox for the duration of this step. Previous series of experiments with a separate sample of Tame foxes showed that this additional positive enforcement was necessary to elicit a peak in emotional arousal, matching the peaks observed at Step 3 in Aggressive and Unselected foxes in response to human enforcement. We can propose that these differences in sensitivity to human enforcement among the three study groups of foxes can be due to the differences in activity of the hypothalamo–pituitary–adrenal (HPA) axis, responsible for the responses to external stimuli. We can propose that these differences in sensitivity to human enforcement among the three study groups of foxes may be due to differences in activity of the HPA axis, responsible for the responses to external stimuli. Strict selection for tame behaviour included a substantial decrease in the levels of adrenocorticotrophic hormone (ACTH) and basal levels of corticosteroids in blood plasma of Tame foxes (Oskina, 1996; Trut, 1999; Trut et al., 2004, 2009; Oskina et al., 2008). As a consequence, the threshold of sensitivity of adrenal glands to ACTH is increased, and the adrenal cortex responds less strongly to emotional stress in Tame foxes (Oskina & Plusnina, 2000; Trut et al., 2004, 2009). Therefore, Tame foxes are apparently less responsive to small changes in behaviour by an experimenter.

Hormonal responses of Tame foxes are very distinctive from both Aggressive and Unselected strains, which are very similar. Specifically, both basal and following a duration of 10-min restraint in human hands, levels of cortisol and ACTH in Aggressive foxes did not differ significantly from those of Unselected foxes. In contrast, in Tame foxes, both basal and ‘post-stress’ levels of cortisol and ACTH were much lower, differing significantly from the values found in Aggressive and Unselected foxes (Oskina et al., 2008). Therefore, we can infer that Aggressive foxes were similarly stressed by human approach as Unselected foxes (Gogoleva et al., 2010a) and experienced

negative emotional arousal during our tests, that was distinctive from Tame foxes.

Different from rodents (Blumstein et al., 2008), there is no reason for silver foxes to use vocal nonlinear phenomena (Wilden et al., 1998; Fitch et al., 2002) as indicators of positive or negative emotional arousal. Previous studies with silver foxes showed that the single call type, where vocal nonlinear phenomena could be found, was whine, occurring across strains and behaviours (Gogoleva et al., 2008, 2009, 2010b). Distinctive to domestic dogs, whines of silver foxes contained only three kinds of nonlinear phenomena, subharmonics, frequency jumps and deterministic chaos, but completely lacked the biphonation that is highly characteristic of canids from the genera *Canis*, *Cuon* and *Lycaon* (Wilden et al., 1998; Volodin & Volodina, 2002; Volodina et al., 2006a,b). In addition, a detailed study of the occurrence of nonlinear phenomena in whines of Aggressive and Tame foxes and their hybrids (Gogoleva et al., 2009) did not reveal significant relationships between aggressiveness and nonlinear phenomena, with the exception of deterministic chaos, that correlated positively with aggressiveness. However, the changes in acoustic quality due to the presence of deterministic chaos in whines was already taken into account by analysis of power parameters of joint calls, so again, a separate analysis of deterministic chaos in whines was redundant in the frames of this study.

In conclusion, Belyaev's silver foxes have proved to be a good model for testing the effects of positive and negative emotional arousal in vocalizations. Compared to domestic dogs, socialized and habituated to humans with different reactions to different people, silver foxes lack personalized relations to people and show their strain-specific behavioural and vocal responses to any human, even when they see him or her for the first time. Furthermore, the previous study by Gogoleva et al. (2010b) showed that foxes of any strain, Tame, Aggressive or Unselected, behave differently toward people compared to conspecifics. Therefore, for our study of silver foxes, humans simply represented stimuli, evoking positive or negative emotions and related vocalizations. In terms of an assessment-management approach (Owings & Morton, 1998) an assessment by the fox was made of the approaching human and a positive or negative stimulus was perceived and responded to accordingly with visual and vocal displays. Here we did not examine management effect of their displays on the human experimenter. However, indirect evidence suggests a strong emotional impact of the emotion-related displays of

silver foxes on humans. Keepers of this experimental farm refuse decisively to work exclusively with Aggressive foxes, apparently because of the arising negative emotional feedback. This is the reason why Tame and Aggressive foxes are not kept in separated cage batteries and share keepers (Gogoleva et al., 2008). Playback research with inexperienced humans as recipients are necessary to examine whether people are able to recognize the emotional content of fox vocalization by sound only, as they are able to do based on vocalization of domestic dogs (e.g., Pongrácz et al., 2006) and domestic cats (*Felis catus*: Nicastro & Owren, 2003), i.e., for the species that have undergone the long-term domestication process.

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