Kind granddaughters of angry grandmothers: The effect of domestication on vocalization in cross-bred silver foxes

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ABSTRACT

The genetic basis of the effects of domestication has previously been examined in relation to morphological, physiological and behavioural traits, but not for vocalizations. According to Belyaev [Belyaev, D.K., 1979. Destabilizing selection as a factor in domestication. J. Hered. 70, 301–308], directional selection for tame behaviour toward humans resulted in domestication. This hypothesis has been confirmed experimentally on the farm-bred silver fox *Vulpes vulpes* population that has undergone 45 years of artificial selection for tameness and 35 years of selection for aggressiveness. These foxes, with their precisely known attitudes toward people, provide a means of examining vocal indicators of tameness and aggressiveness to establish the genetic basis for vocal production in canids. We examined vocalizations toward people in foxes selected for tameness and aggressiveness compared to those of three kinds of crosses: Hybrids (Tame × Aggressive), A-Backcrosses (Aggressive × Hybrid) and T-Backcrosses (Tame × Hybrid). We report the effects of selection for tameness on usage and structure of different vocalizations and suggest that vocal indicators for tameness and aggressiveness toward people are discrete phenotypic traits in silver foxes.

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1. Introduction

A number of morphological and physiological changes have proved to be related to domestication (Darwin, 1868; Hediger, 1964; Belyaev, 1979; Trut, 1999, 2001). Directional selection for tameness and against fearfulness towards humans resulted in the appearance of traits such as white patches, floppy ears and curled tails in many unrelated species of domesticated animals (Belyaev, 1979), and may affect also vocalization (Cohen and Fox, 1976; Budde, 1998; Monticelli and Ades, 2001; Nicastro and Owren, 2003). Nevertheless the effects of domestication on vocal traits are still poorly understood.

Evolution of the domestic dog *Canis familiaris* from the timber wolf *Canis lupus* represents an example of the transition from wildness to tameness. Presumably domestic dog vocalizations evolved from their timber wolf ancestors during the period of their independent evolution (Tchernov and Valla, 1997; Sablin and Khlopachev, 2002), but their vocal repertoires may have already differed at the start of domestication. So comparing the wolf and dog calls would not provide much insight into how domestication affects vocalization. Domesticated Belyaev's silver foxes *Vulpes vulpes* (Belyaev, 1979) still offer an unique opportunity to study the effects of domestication because the foxes, unlike dogs and wolves, have detailed strains and the process of selection was completely artificial and well recorded, giving scientists access to detailed information on exactly how these groups differ as well as the ability to cross and back cross to different strains.

Farm silver foxes normally show aggressive–fearful responses to humans. The founder population has undergone an artificial directional selection for tame behaviour (Tame foxes), started in 1960 (e.g. Trut, 1999), and similar selection for aggressive–fearful behaviour (Aggressive foxes), started in 1970 in Novosibirsk (Russia) (Kukekova et al., 2008). Throughout selection for aggression, both the degree of aggression and the degree of fearfulness to humans have been scored. The fearful individuals have been repeatedly excluded from breeding, and in current generations of Aggressive foxes, aggressiveness significantly prevails over fearfulness. They are not afraid of humans, do not try to increase their distance from an approaching human and instead tend to attack him. Cross fostering, cross breeding and embryo transplantation experiments have shown that behavioural differences between Tame and Aggressive foxes are genetically determined (Trut, 1980, 2001). In addition, a

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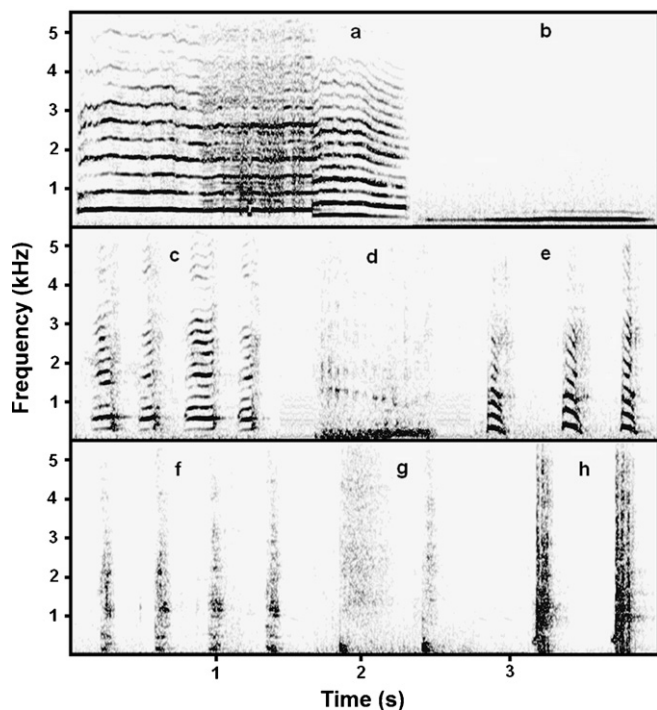*E-mail address:* volodinsvoc@gmail.com (I.A. Volodin).

Fig. 1. Spectrogram illustrating call types produced toward people by silver foxes: (a) *whine*, (b) *moo*, (c) *cackle*, (d) *growl*, (e) *bark*, (f) *pant*, (g) *snort*, (h) *cough*. Calls for figure spectrograms were digitized with 11.025 kHz sampling frequency; spectrograms were created using Hamming window, FFT-length 512 points, frame 50%, and overlap 87.5%. Supplementary audio illustrates the eight call types whose spectrograms are presented here.

population of foxes whose behaviour has not been selected (Unselected foxes) has been living on this farm (Trut, 1999; Kukekova et al., 2008).

Supplementary Movies 1–3 illustrate the typical behavioural responses of Unselected, Aggressive and Tame foxes to an unfamiliar researcher (SG), when they met her for the first time in their lives. The Unselected fox with its wild type behaviour toward people enlarged the animal-human distance and showed escape responses. The Aggressive fox shortened the animal–human distance and attacked. Finally, the Tame fox approached the researcher and wagged its tail.

Belyaev's foxes, with over 45 years of selective breeding for tameness and 35 years of similar selection for aggressiveness, represent a valuable resource for the scientific world. The study of foxes differing in tameness may help to estimate both quantitative and qualitative shifts in vocal behaviour that have occurred under domestication. Since the attitudes of Tame and Aggressive foxes toward people are precisely known, this fox model allows us to understand which vocal traits are specifically related to aggression or tameness toward humans.

Many aspects of this population have been studied in depth, including their genetics (Trut, 1980; Kukekova et al., 2004, 2006, 2007; Lindberg et al., 2007), behaviour (Plyusnina et al., 1991; Trut, 1999; Trut et al., 2004; Hare et al., 2005; Kukekova et al., 2008), physiology (Belyaev and Trut, 1983; Popova et al., 1991; Oskina, 1996; Osadchuk, 1999; Trut et al., 2000; Gulevich et al., 2004) and morphology (Trut et al., 1991, 2006). In a recent study (Gogoleva et al., 2008a) we compared vocalizations of Tame and Aggressive strains of foxes with those of Unselected foxes. In response to humans foxes produced five voiced, or tonal (*whine, moo, cackle, growl* and *bark*) and three unvoiced, or noisy (*pant, snort* and *cough*) call types (Fig. 1). Voiced calls showed signs of production from a vocal source (i.e., larynx with vocal folds): a tonal spectrum with

a fundamental frequency and harmonics, sometimes complicated with nonlinear phenomena and/or articulation effects. Unvoiced calls lacked the fundamental frequency and had an explosive wideband spectrum, revealing their non-vocal nature, i.e., production not with vocal folds but from another source, most probably turbulence (vortices), occurring during the passage of air through a narrowest part of vocal tract. Traits used to classify fox calls into eight types, are described in Gogoleva et al. (2008a). Supplementary audio illustrates the sound of the eight call types, whose spectrograms are given in Fig. 1.

Call types were the same in Aggressive and Unselected foxes, suggesting that selection for aggression does not affect vocal behaviour. At the same time, Tame foxes used distinctive call types, suggesting that the selection for tame behaviour affects vocalization. The *snort* and *cough* were specific to Aggressive and Unselected foxes and have proved to be vocal indicators of aggressiveness. The *cackle* and *pant*, on the other hand, were specific to Tame foxes and have proved to be vocal indicators of tameness. The *whine* occurred in all the three fox strains and therefore was unaffected by any selection for behaviour. The strain-specific vocalization was consistent between different fox samples and between generations (Gogoleva et al., 2008b).

While the effects of directional selection by behaviour on vocalization have been studied for the extreme forms, i.e. for the Tame and Aggressive strains compared to the Unselected control foxes (Gogoleva et al., 2008a,b), these effects are not yet understood for the intermediate cross forms, that are graded in tameness and aggressiveness. The purpose of this study was to investigate the relationships between the tameness and call usage and between the tameness and acoustic structure of *whines* in greater detail. Specifically, we compared vocalizations directed toward people between Tame foxes, Aggressive foxes and three kinds of crosses between them. For *whines*, we investigated the occurrence of nonlinear phenomena and articulation effects, to understand their role as vocal indicators of aggressiveness and tameness.

2. Materials and methods

2.1. Subjects, site and dates of work

Our subjects were 125 adult female silver foxes, aged from 1 to 2 years, kept at the experimental fur farm of the Institute of Cytology and Genetics, Novosibirsk, Russia. Five study groups, 25 individuals per group, included Tame (selected for tameness, 44–45 generations since the start of selection), Aggressive (selected for aggressiveness; 34–35 generations since the start of selection), Hybrid (cross Tame × Aggressive), A-Backcross (cross Aggressive × Hybrid), and T-Backcross (cross Tame × Hybrid) foxes. We used only female foxes, because we did not have had the necessary sample of males (the sex ratio in industrial fur populations is usually 1:4 in favor of females).

Foxes were kept in individual outdoor cages, consisting of two compartments, one 70 cm × 85 cm × 90 cm with wire mesh floor and another with wooden shelter 70 cm × 85 cm × 85 cm, with sawdust bedding. 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 byproducts, minced chicken, cereals, vitamins and minerals). Bones were given as food and play enrichment. Water was available *ad libitum*.

The fox calls were recorded in July–August 2005 and 2006 in their individual outdoor wire mesh cages. Foxes from different

study groups were kept mixed; no group was kept in separated batteries from others. On this farm, it is prohibited to pet any particular fox, because it may influence on behaviour of animals. 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 2 months. After separation they remain in visual, olfactory and auditory contact with foxes from neighboring and opposing cages. This holding regime has been kept standard since the start of Belyaev's experiment and is uniform for all foxes on this farm, thereby excluding the influence of any additional factors on the behaviour of the animals.

2.2. Behaviour scores

At puberty (7–8 months) all study foxes were tested for behavioural responses towards humans with a standard test, which was developed at the onset of behavioural selection and has been applied to all individuals on the experimental farm except Unselected foxes, to whom this test is not administered (Belyaev, 1979; Trut, 1980, 1999; Kukekova et al., 2008). The test procedure includes the approach of a human experimenter to the cage of a focal fox followed by opening the cage door and an attempt to caress it. According to its non-vocal behavioural responses during this test, each fox received a "behavioural score". The score can vary between −4 (extreme aggressiveness) to +4 (extreme friendliness). At early stages of Belyaev's experiment, the scale counted eight integer scores (from −4 to +4). Later, additional intermediate scores were added for more detailed estimation of fox behaviour on the scale aggressiveness–friendliness. Currently the scale counts these 22 gradations and all foxes of this study were tested according to it. Mean + SD behaviour score for the Aggressive study group was: −1.5 ± 0.86; for A-Backcross: −0.36 ± 1.66; for Hybrid: 0.76 ± 1.85; for T-Backcross: 2.38 ± 1.21; and for Tame: 3.63 ± 0.25.

2.3. Data collection

We used a Marantz PMD-222 (D&M Professional, Kanagawa, Japan) cassette recorder with an AKG-C1000S (AKG-Acoustics Gmbh, 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.

All acoustic recordings (one per animal) were done by the same researcher (SG), unfamiliar to the foxes. SG approached the cage of the focal fox up to a distance of 50–100 cm. Recording started when the fox started calling and lasted 4–6 min. A threshold for calling varied between individuals: if an animal did not vocalize spontaneously in response to the human approach, the researcher provoked vocalization by moving a hand toward the cage, opening the cage door or by caressing the animal. As soon as the animal started vocalizing, the stimulation was stopped. The distance between the microphone and a focal fox varied of 25–100 cm; the orientation of an animal to the microphone was mostly frontal or lateral. If a non-focal fox called simultaneously with the focal one, the calls of the focal fox were labeled by voice (as in Supplementary Movie 3). The labeling of calls by voice is a traditional practice, inevitable when a few animals call simultaneously. It allows distinguishing between calls of focal and other animals during the following analysis.

2.4. Call analysis

Calls were analyzed using Avisoft SASLab Pro software v. 4.33 (Avisoft Bioacoustics, Berlin, Germany). Call recordings were digitized at 22.05 kHz sampling frequency and 16-bit precision, each

recording as a separate file. Spectrograms for analysis were created using Hamming window, FFT-length 1024 points, frame 50%, and overlap 87.5%. These settings provided a bandwidth of 56 Hz, frequency resolution of 21 Hz and time resolution of 5.8 ms. One observer (SG), classified each call visually to one of eight structural types (Fig. 1), blindly to the strain to which a fox belonged to. We considered sound utterances as separate calls if they were separated by a silent interval longer than 20 ms. In total, we examined 25,527 calls.

To calculate the time spent vocalizing (i.e., the total duration of all calls produced by a focal fox during a recording session), we measured the duration of each recording session and the duration of each call with the standard marker cursor in the main window of Avisoft. The measurements were exported automatically to Excel (Microsoft Corp., Redmond, WA, USA). To calculate the calling rate for each call type, we divided the number of calls of the given type by the duration of the recording session (in minutes).

For calls assigned to the type *whine*, we scored the presence/absence of nonlinear phenomena: frequency jumps, subharmonics and deterministic chaos (Wilden et al., 1998; Fitch et al., 2002), using the same approach as previously applied to domestic dogs (Volodina et al., 2006a). None of the whines contained biphonation (Gogoleva et al., 2008a), the forth nonlinear phenomenon, occurring regularly in canids from genus *Canis*, *Cuon* and *Lycaon* (e.g. Wilden et al., 1998; Volodin and Volodina, 2002; Volodina et al., 2006a,b). We scored all prominent frequency jumps within calls. Also, we scored the appearance of subharmonics and/or deterministic chaos in cases where the total duration of the call portions bearing these nonlinear phenomena was at least 30 ms for calls shorter than 300 ms, not less than 10% of duration for calls with duration of 300–1000 ms, and at least 100 ms for calls longer than 1000 ms.

For *whines*, we also scored the presence of articulation effects. Flutter was scored if two or more inverted-U modulations of the fundamental frequency, one after another, were found in a call. Rhythm was scored if two or more successive sound pulses, breaking a tonal vocalization, occurred in a call. Babble was scored if at least one U-shaped modulation of the fundamental frequency was present in a call (for details of measurements of nonlinear phenomena and articulation effects, see Gogoleva et al., 2008a). For each individual, we calculated the proportion of *whines* containing each type of nonlinear phenomena and/or articulation effects.

More than one nonlinear phenomenon and/or articulation effect could occur within a *whine*. Each *whine* was judged to include or not include each of the events in a zero-one fashion (Riede et al., 2004; Volodina et al., 2006a). In total, we examined 11988 *whines* of 117 foxes.

2.5. Statistical analyses

All the analyses were carried out with STATISTICA, v. 6.0 (StatSoft, Inc., Tulsa, OK, USA). All tests were two tailed; all means are given as mean ± SE. We used one-way ANOVAs with Tukey post hoc tests to compare time spent vocalizing (total duration of calls within a recording divided by the duration of the recording) among the five study groups, as the distribution of values for time spent vocalizing did not differ from normality in all the five study groups ($P > 0.05$, Kolmogorov–Smirnov test). We used Fisher exact test to compare the proportions of foxes producing each call type in the five study groups. We used Spearman rank correlation with Bonferroni corrections to estimate correlations between individual behaviour scores and individual calling rates for particular call types, as well as between individual behaviour scores and the occurrence of nonlinear phenomena and articulation effects in *whines*.
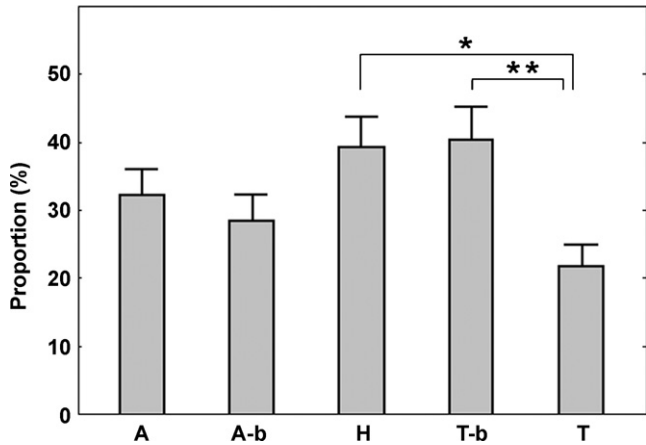
Fig. 2. Mean ± SE proportion of time spent vocalizing per each study group: A—Aggressive, A-b—A-Backcross, H—Hybrid, T-b—T-Backcross, T—Tame and the differences between the groups revealed with ANOVA followed by Tukey post hoc test: **$P < 0.01$; *$P < 0.05$.

3. Results

A one-way ANOVA, with factor study group, showed significant differences in time spent vocalizing between the five study groups ($F_{4,120} = 4.3$, $P = 0.003$). The proportion of time spent vocalizing was the lowest in Tame foxes, highest in T-Backcross and Hybrid foxes, and intermediate in Aggressive and A-Backcrosses (Fig. 2). Tukey post hoc tests revealed significant differences between Tame and Hybrid ($P = 0.01$) and between Tame and T-Backcross study groups ($P = 0.006$).

Call type sets were distinctive between the study groups. Only *whines*, *moos* and *growls* occurred in all the five study groups (Fig. 3). Aggressive and A-Backcross foxes never cackled or panted, while Tame and T-Backcrosses (except one individual) never coughed or snorted. *Barks* were the rarest vocalizations, recorded only from two Aggressive and five A-Backcross foxes. Hybrids were the only fox group which used the whole set of the eight call types, except the *bark* (Fig. 3).

To estimate the study group effect on the occurrence of a particular call type, we compared, with Fisher exact test, the numbers of foxes producing the given call type within a group, with the mean value (the number of foxes producing the given call type divided by the number of study groups, i.e. five). The two groups which were the most distinctive in their attitudes toward people (Aggressive and Tame) were also the most distinctive in use of five of the eight call types (Fig. 3). Numbers of Aggressive individuals producing *snorts*, *coughs* and *moos*, were significantly higher the mean value, whereas the numbers of Tame foxes producing each of these three call types, were significantly lower than the mean value. Numbers of Tame foxes producing *cackles* and *pants* were significantly higher then the mean value, whereas the numbers of Aggressive foxes producing each of these two call types were significantly lower the mean value. Numbers of individuals, producing *whines*, *growls* and *barks* in the Aggressive and Tame groups did not differ from the mean value. For the Hybrid group, the number of animals producing any call type did not differ significantly from the mean value, and this group was intermediate between Tame and Aggressive groups. The A-Backcross group did not differ from the Aggressive group. The number of individuals from the T-Backcross group uttering *cackles*, *pants* and *moos* did not differ from the mean value, and this group was intermediate between the Hybrid and Tame groups (Fig. 3).
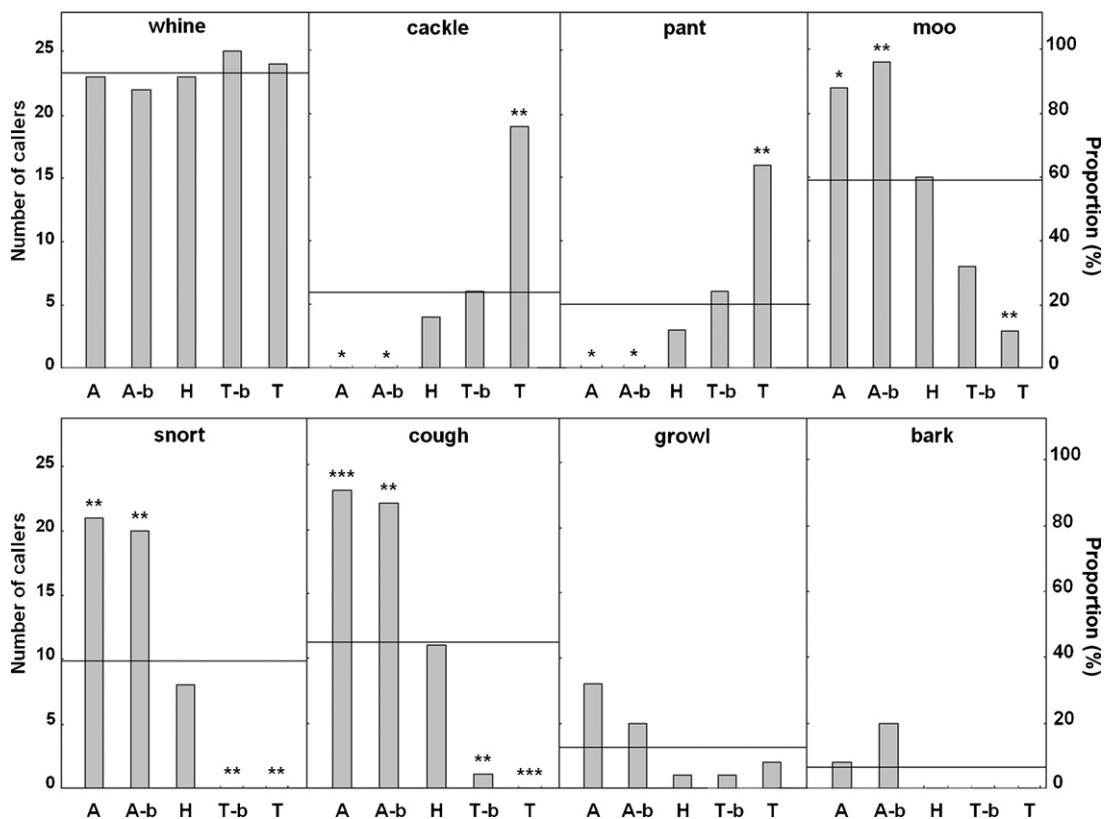

Fig. 3. Number and proportion of foxes that produced particular call types in each study group: A—Aggressive, A-b—A-Backcross, H—Hybrid, T-b—T-Backcross, T—Tame and the results of comparisons between the observed and mean values for numbers of individuals producing the given call type (horizontal lines) with Fisher exact test: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.
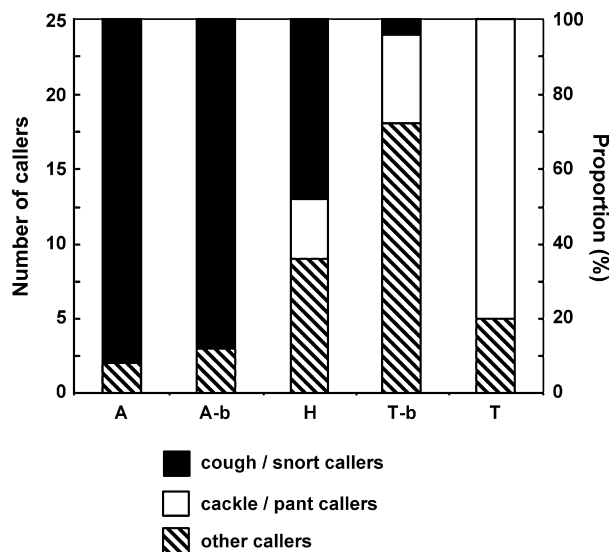
Fig. 4. Number and proportion of *cough*/*snort* callers (i.e. foxes producing vocal types indicative of aggressiveness) *cackle*/*pant* callers (i.e. foxes producing vocal types indicative of tameness) and other callers (i.e. foxes, which lacked either *cough*/*snort* or *cackle*/*pant*, but still called other call types) for each study group: A—Aggressive, A-b—A-Backcross, H—Hybrid, T-b—T-Backcross, T—Tame.

Table 1
Spearman rank correlation coefficients (r_s) between individual fox behaviour scores (BS) and calling rate (calls/min) for each call type, proportion of *whines* containing nonlinear phenomena and proportion of *whines* containing articulation effects.

Correlation between	r_s	P
BS and calling rate (N = 125)		
All call types	0.026	0.77
Whine	0.007	0.94
Growl	−0.295	**<0.001**
Moo	−0.554	**<0.001**
Snort	−0.613	**<0.001**
Cough	−0.668	**<0.001**
Cackle	0.669	**<0.001**
Pant	0.610	**<0.001**
Bark	0.064	0.48
BS and articulation effect (N = 117)		
Any articulation effect	−0.312	**<0.001**
Rhythm	−0.455	**<0.001**
Babble	−0.430	**<0.001**
Flutter	0.289	**0.002**
BS and nonlinear phenomena (N = 117)		
Any nonlinear phenomenon	−0.263	**0.004**
Frequency jump	0.135	0.15
Subharmonics	0.011	0.91
Deterministic chaos	−0.327	**<0.001**

N = number of animals, P estimates less than 0.0056 (after Bonferroni correction for BS and calling rate) and less than 0.0125 (after Bonferroni correction for BS and articulation effect and for BS and nonlinear phenomena) are shown in bold.

We examined the occurrence of vocal types suggested previously (Gogoleva et al., 2008a) as indicators of tameness (*cackle* and *pant*), and indicators of aggressiveness (*cough* and *snort*), in all the 125 study foxes. We did not find any single fox that used both the *cackle*/*pant* and *cough*/*snort*. *Cackles* and/or *pants* occurred in 30 (24%) individuals (*cackle*/*pant* callers), and *coughs* and/or *snorts*—in 58 (46%) individuals (*cough*/*snort* callers). The remaining 37 (30%) foxes (other callers) lacked these indicator call types.

The numbers and proportion of callers producing the indicator call types differed strongly between the five study groups (Fig. 4). Most of *cough*/*snort* callers were from the Aggressive and A-Backcross groups, whereas most of *cackle*/*pant* callers were from the Tame group. Most of other callers belonged to T-Backcross group, and this group also contained one *cough*/*snort* caller. Thus, the A-Backcross study group was similar to the Aggressive group with respect to call type use. There was little similarity between T-Backcross and Tame study groups with respect to call type use (Fig. 4).

For individual foxes, we found no correlation between the calling rate of all call types and the behaviour score (Table 1). We did find a significant negative correlation between calling rates separately for *cough*, *snort*, *moo* and *growl* and the behaviour score and a significant positive correlation between the calling rates separately for *cackle* and *pant* and the behaviour score. For *barks* and *whines*, no correlations were found (Table 1).

Whines occurred in all the five study groups (Fig. 3). For individual foxes, we found a significant negative correlation between the behaviour score and proportion of *whines* containing the nonlinear phenomena or articulation effects (Table 1). However, among *whines* with nonlinear phenomena, only percentage of *whines* with deterministic chaos showed a significant negative correlation with the behaviour score, whereas percentages of *whines* containing either frequency jumps or subharmonics did not correlate with it. Among articulation effects, percentages of *whines* either with rhythm or with babble showed a significant negative correlation with the behaviour score, whereas the percentages of *whines* with flutter did not correlate with it (Table 1).

4. Discussion

Only certain call types were related to aggressive or tame attitudes of foxes to people. The *cackle* and *pant* occurred more often than average only in the Tame study group and their occurrence correlated positively with individual behaviour scores. *Cough* and *snort* occurred more often than average in the Aggressive and A-Backcross study groups, and their occurrence correlated negatively with individual behaviour scores. Thus the four call types represent vocal indicators of tameness and aggressiveness toward people in the silver fox. Call types indicative of aggressiveness substituted progressively with call types indicative of tameness in the order Aggressive–A-Backcross–Hybrid–T-Backcross–Tame. The Hybrid was the only study group in which all the four indicator call types occurred within a group.

The call types indicating tameness (*cackle* and *pant*) and those indicating aggressiveness (*cough* and *snort*) appeared to be discrete phenotypic traits. Among cross-breed study groups (A-Backcrosses, Hybrids and T-Backcrosses), individual foxes used *cackle*/*pants* or *cough*/*snorts*, but no fox combined the vocal indicators for tameness with those for aggressiveness within their vocal type set. Therefore, the cross-breed study groups differed in the proportion of foxes producing vocal indicators of aggressiveness and vocal indicators of tameness. These findings are consistent with the results of tests for behavioural scores: Hybrid foxes perform either tame or aggressive behaviour toward people, and very rarely display intermediate attitudes to them (Trut, 1980; Plyusnina, 1991).

A single call type occurring in most individual foxes throughout the study groups and not related to tameness or aggressiveness was the *whine*. However, the dimension tameness/aggressiveness was found to be related to the internal structure of this very variable vocalization. The occurrence of the articulation effects rhythm and babble and of the nonlinear phenomenon deterministic chaos showed significant negative correlations with the individual behaviour score. This suggests a relationship between tameness/aggressiveness and sound production. Voiced (tonal) calls, such as fox *whines*, in all terrestrial mammals are produced with phonation which is based on vibrations of the vocal folds (Titze, 1994). Unvoiced noisy calls, like fox *coughs* and *snorts*, are

due to turbulence resulting from the flow of the air through the narrow vocal tract, after passing through the focal folds (in the nasal cavity for the *snort* and in the oral cavity for the *cough*) (Fant, 1960; Gogoleva et al., 2008a). Unlike the nonlinear phenomena, that result from the work of the vocal folds (Wilden et al., 1998; Fitch et al., 2002), articulation effects result from the action work of the vocal tract above the vocal folds Fant, 1960; Titze, 1994). We can thus hypothesize that aggressiveness toward people elicits tension in certain muscles (Zahavi, 1982), which influence the shape of vocal tract and the action of articulators (tongue, mandible, soft palate). Findings that the call structures are indicators of aggressiveness are in accordance with motivational–structural rules, suggesting the low-frequency noisy structure for calls occurring in aggressive contexts (Morton, 1977).

While in the present study the time spent vocalizing was the lowest in Tame foxes, a prior study (Gogoleva et al., 2008a) found that the group that spent the lowest time vocalizing was the Unselected foxes. Unselected foxes vocalized only 10.3% of the time, significantly less than either the Tame or Aggressive foxes. Thus those foxes that were selected by behaviour (Tame and Aggressive), spent at least twice as much time in vocalization compared to foxes with wild type behaviour (Unselected) (Gogoleva et al., 2008a). Our data thus support Cohen and Fox's (1976) hypothesis about the relaxation of selection pressure for silence with domestication in canids. In wild foxes, silence may be important to prevent the frightening of potential prey. Another relevant hypothesis here, after Nicastro and Owren (2003), is that increased time spent in vocalization during domestication resulted from implicit selection by humans, who provided more care to more active callers. For instance, human listeners recognize the contexts in meow bouts better than in single meows in domestic cats *Felis catus* (Nicastro and Owren, 2003).

The T-Backcrosses were more distinct from Tame foxes than A-Backcrosses were from Aggressive foxes. This might be related to different effects of selection for tameness and aggressiveness on basic cortisol levels in foxes. The selection for fearlessness and tameness resulted in a drastic fall of the basal cortisol level, which was four times lower than in Unselected foxes (e.g. Trut, 1999; Trut et al., 2004). In contrast, the selection for the fearlessness and aggressiveness did not affect the endocrine system, with basal cortisol levels remaining undistinguishable between Aggressive and Unselected foxes (Gulevich et al., 2004). These findings are consistent with our previous data (Gogoleva et al., 2008a): the selection for aggressive behaviour did not affect fox vocal behaviour, whereas the selection for tame behaviour resulted in drastic changes in usage of calls produced toward people compared to the Unselected wild type. Similarly, in their non-vocal behaviour traits, Aggressive foxes are very close to the Unselected wild type, whereas Tame foxes are distinctive from them (Kukekova et al., 2008).

Tame foxes did not *bark* in response to human approach. This lends some doubt to the hypothesis that barking has evolved as a special vocalization for communication between dogs and humans (Feddersen-Petersen, 2000; Yin, 2002). Instead, the *bark* was elicited in two Aggressive and five A-Backcross individuals, i.e. in foxes whose vocal behaviour is close to wild type foxes (Gogoleva et al., 2008a). In the wild, red foxes also produce *barks*, but contexts of emission of this call type are not established to date (Newton-Fisher et al., 1993). The fact that *barks* did not occur in Tame foxes, but did occur in Aggressive and A-Backcrosses suggest that the enhanced level of barking is the effect of selection for fearlessness and aggressiveness rather than the effect of selection for tameness.

Unlike domestic dogs, Tame foxes interacted with humans with *cackles* and *pants*. Swift foxes *Vulpes velox* and Arctic foxes *Alopex lagopus,* which are closely related to the red fox, use the *cackle* for communication with their pair mates and pups (Ovsjanikov et al., 1988; Darden and Dabelsteen, 2006). Domestic dogs use the *bark*

and *whine* to interact with humans (Yin, 2002; Yin and McCowan, 2004; Volodina et al., 2006a). Therefore, the use of a certain call type for communicating with humans might depend not only on domestication, but also on the species.

Acknowledgements

Appendix A. Supplementary data

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