Current Zoology, 2020, 66(4), 407–415
doi: 10.1093/cz/zoz055
Advance Access Publication Date: 12 November 2019
Article

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Geographic variability in the alarm calls of the European ground squirrel

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Handling editor: Zhi-Yun Jia

Received on 2 June 2019; accepted on 9 November 2019

Abstract

Geographic variability in vocalizations has been documented in many mammalian species. We examined to what extent it applies to the alarm calls of the European ground squirrel *Spermophilus citellus*. We recorded the calls of 82 adult individuals from 5 natural colonies in the Czech Republic and 24 adult individuals from an artificial seminatural colony located in a Czech zoo. The founders of this colony originated from 4 different natural colonies in the Czech Republic. Our results showed that there are hardly any differences in the acoustic structure of the alarm calls between male and female European ground squirrels. Discriminant function analysis showed the highest degree of discriminability for the most isolated sites (54–74% of individuals classified correctly), whereas the lowest degree of discriminability was found for 2 interconnected colonies (38–40% individuals classified correctly). Individuals from the artificial seminatural colony were often classified correctly to this colony (58% classified correctly); however, the precision of the classification was comparatively relatively low, that is, many individuals from other colonies were incorrectly classified into this seminatural colony. This likely corresponds to the different origins of its founders. These findings indicate that there is a rather substantial geographic variability in the alarm calls of the European ground squirrel, and our study highlights its possible impact on conservation measures such as establishing artificial colonies or reintroductions.

Key words: artificial population, intraspecies variability, mammalian vocalization, Rodentia, *Spermophilus citellus*

Geographic variability in intraspecific acoustic signals, which is manifested as larger differences among geographically isolated populations rather than within these populations, has been documented for many invertebrate as well as vertebrate taxa (e.g., Gerhardt and Huber 2002; Kroodsma 2004). Different mechanisms, particularly natural and sexual selection, cultural transmission and genetic drift (Campbell et al. 2010; Wilkins et al. 2013), have been proposed to contribute to this intraspecific variability (Wilkins et al. 2013), with several of these mechanisms possibly contributing at the same time in some taxa (Slobodchikoff and Coast 1980; Wilkins et al. 2013).

Alarm calls of ground-dwelling sciurids represent one of the most intensively studied mammalian vocalizations (Blumstein 2007). These acoustic signals emitted in the presence of a perceived danger are highly species-specific (Blumstein 2007), and intraspecific geographic variability has also been documented in a variety of species, particularly in prairie dogs (Perla and Slobodchikoff 2002), marmots (Nikol'skii et al. 1999), and ground squirrels (Eiler and Banack 2004; Matrosova et al. 2016). However, the mechanisms contributing to the intra- and interspecific variability are not yet fully understood. Studying the geographic variation in the alarm calls of ground-dwelling sciurids is therefore beneficial for revealing the mechanisms that contribute to the evolution and divergence of these acoustic signals (Wilkins et al. 2013). Additionally, knowing and understanding the patterns of geographic variation in animal vocalizations may also be useful from the conservation perspective. The possible impact of acoustic diversity on the successful reintroductions or establishment of novel populations started to be investigated quite recently (Magroski et al. 2017; Martins et al. 2018).

The European ground squirrel *Spermophilus citellus* is a diurnal colonial rodent. Although it was formerly continuously distributed across the shortgrass steppes of Central and Southeastern Europe, its distribution area is currently facing considerable reduction and fragmentation (Matějů et al. 2010b). A gradual decline in this species has been documented in the Czech Republic since approximately the beginning of the 1960s, resulting in the existence of ~38 highly fragmented small populations across the country in 2018 (Jan Matějů and Jitka Větrovcová, unpublished data). Most of these populations occur in artificial habitats with maintained low grass cover (e.g., airfields, sports fields, and campsites). A study on the population genetic structure of the European ground squirrel showed that there is a strong genetic differentiation among the Czech populations and typically a high level of inbreeding within populations (Hulová and Sedláček 2008). The species is listed as critically endangered in the Czech Republic and vulnerable in Europe (Anděra and Červený 2003; Coroiu et al. 2008). Therefore, several conservation measures have been adopted to prevent the potential extinction of this rodent species in the Czech Republic and elsewhere (Matějů et al. 2010a). These measures include the establishment of migration corridors, which allow connection and gene flow between populations, and establishment of semicaptive colonies as a source of animals for later repatriations and reintroductions (Matějů et al. 2010a). Thus, the current distribution of the European ground squirrel in the Czech Republic (Figure 1) provides a unique opportunity to explore the geographic variability in its alarm calls.

As a typical representative of the ground-dwelling sciurids, the European ground squirrel emits alarm calls in the presence of a potential danger and sometimes emits them in a long series as long as the danger persists (Schneiderová 2012). The alarm call of this species consists of 2 tonal, structurally different syllables. The first syllable has an almost constant fundamental frequency of ~8 kHz, whereas the fundamental frequency of the second syllable is modulated by ~12 kHz (Schneiderová and Policht 2012). Alarm calls comprising only the first element are also produced frequently, and the possible adaptive significance of this phenomenon has not yet been clarified. Repeated recordings of permanently marked European ground squirrels revealed that the alarm calls containing both syllables retain their individualistic acoustic structure for longer periods than alarm calls containing only the first syllable (Schneiderová et al. 2017).

Previous research has shown that alarm calls of the European ground squirrel manifest strong short-term individual distinctiveness (Schneiderová and Policht 2010; Schneiderová et al. 2017). Discriminant function analysis (DFA) classified alarm calls recorded from 8 animals during a single recording session per individual with an accuracy of almost 98% (Schneiderová and Policht 2010). The aim of this study is to further examine the intraspecific variability in the alarm calls of the European ground squirrel. In particular, we aimed to determine whether there is a geographic variability and which acoustic parameters mostly contribute to the differentiation

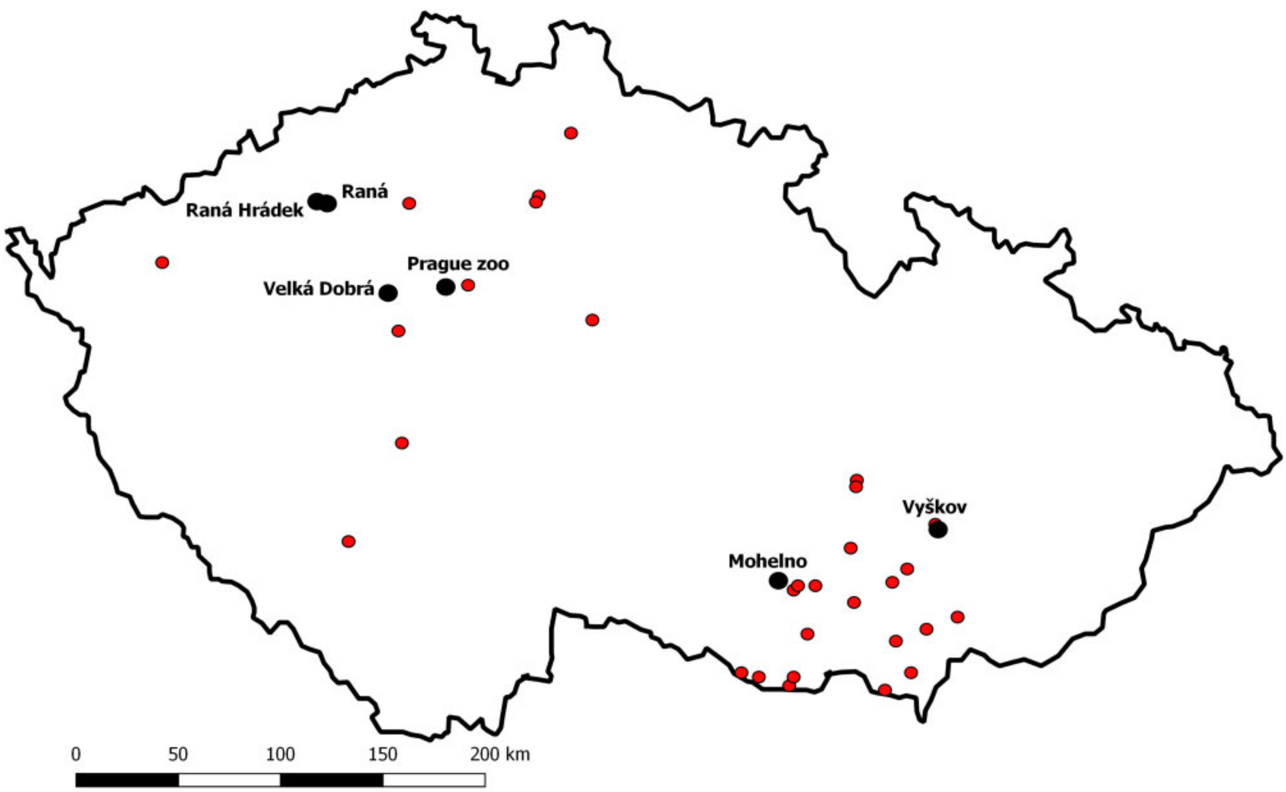

Figure 1. A map showing the distribution (red and black points) of the European ground squirrel in the Czech Republic in 2017. The study sites are shown in black color and labeled.

among the studied populations. We further aimed to test whether there are sex-related differences in the alarm calls of this rodent species. Examining all these levels of intraspecific variability will help us to understand acoustic communication and antipredatory strategy of this rodent and the knowledge can also be beneficial when managing populations of this endangered species.

Materials and Methods

Study sites and animals

The alarm calls of adult European ground squirrels were recorded at 5 natural colonies and 1 seminatural colony located in the Czech Republic (Table 1; Figure 1). The natural colonies included Raná and Raná Hrádek, which are located within 600 m of each other, and continuous contact of the animals has been documented between them. A migration corridor was established between 2009 and 2013 to enhance the contact between these 2 colonies. Additional natural colonies were found in Mohelno, Vyškov, and Velká Dobrá, which have been separated and isolated from each other as well as from the colonies in Raná and Raná Hrádek since the 1970s (Hulová and Sedláček 2008; Matějů et al. 2008). The colony in Velká Dobrá should be considered natural with caution. European ground squirrels were probably introduced to this airfield by local staff; 10 introduced individuals were most likely taken from a single colony located at Bořitov (airfield, N 49° 26′ 9.89″, E 16° 35′ 38.99″; Matějů et al. 2010b). The seminatural colony was located at the Prague Zoo, where it was established in 2006 as a potential source of animals for later reintroductions. The founders of the colony were 73 individuals (33 males and 40 females) originating from 4 natural colonies located in the Czech Republic (Raná; Raná Hrádek; Praha-Letňany, airfield, N 50° 7′ 52.98″, E 14° 31′ 31.99″; Mladá Boleslav–Bezděčín, airfield, N 50° 23′ 53.82″, E 14° 53′ 54.52″). These founders were introduced gradually to the Prague Zoo colony between 2006 and 2011. More details about this colony can be found in Schneiderová et al. (2015).

Data collection

The recordings of adult (>1 year) European ground squirrels' alarm calls were collected from 8 AM to 7 PM during June or July when the current year's juveniles had already emerged from their maternal burrows. The emission of alarm calls by this species increases during this time of their active season, which lasts from approximately March to August (Katona et al. 2002). The recorded alarm calls were emitted by live-trapped animals that spontaneously produced them toward a researcher who was slowly walking at a short distance (1–3 m) from the trap. The traps were constructed from wire mesh and baited with apples and carrots. Live-trapped ground-dwelling sciurids typically emit alarm calls that are indistinguishable from those emitted by free-ranging animals (Matrosova et al. 2010); therefore, this method is generally used for their recordings (Hanson and Coss 2001; Blumstein and Daniel 2004; Matrosova et al. 2009). A Marantz PMD-661 solid-state recorder (D&M Professional, Kanagawa, Japan) with internal microphones was used to record the alarm calls. The settings of the recorder (recording format 16-bit, sampling rate 44.1 kHz, frequency response 20–20000 Hz, −18 dB gain on the internal microphones) were kept standardized throughout the data collection. Each animal was removed from the trap after the recording, and its sex was determined.

Acoustic analysis

The Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany) was used for the detailed acoustic analysis. A minimum of 4 and a maximum of 10 alarm calls consisting of both syllables were selected per individual. The alarm calls were visualized with a spectrogram with the following settings: sampling rate 44.1 kHz, Hamming window, FFT length 512 points, frame size 50%, and overlap 93.75%. The "standard eraser cursor" and the "remove erased spectrogram sections from waveform" tools were used to edit all the alarm calls, so they comprised the fundamental harmonic (*f0*) only. The "automatic parameter measurements" tool was used to measure 10 acoustic parameters from the fundamental harmonic (*f0*) for each of 2 syllables (Figure 2): the duration of the syllable (*dur*), distance from the start to the maximum amplitude of the syllable (*dstm*), starting frequency of the syllable (*start*), ending frequency of the syllable (*end*), center frequency of the syllable (*center*), frequency with the maximum amplitude of the syllable (*maxamp*), mean frequency of the syllable (*mean*), the relative standard deviation (SD) of the syllable (*std*), minimum frequency of the syllable (*min*), and maximum frequency of the syllable (*max*). The last 2 parameters were extracted by the software as a minimum and maximum frequency of the entire syllable with a threshold value of −20 dB. The relative SD of the syllable (*std*), used in this study to quantify frequency modulation (Specht 2004), was extracted by the software as the SD/mean value of the frequencies computed for all the spectra between the start and the end of the syllable.

Table 1. Summary of the natural (Raná, Raná Hrádek, Mohelno, Vyškov, and Velká Dobrá) and seminatural (Prague Zoo) colonies where the alarm calls were recorded from 2011 to 2017

Colony	Coordinates	Data collection	Estimated abundance	Number of individuals recorded		
				Males	Females	Total
Raná (Raná National Nature Reserve)	N 50° 24′ 24.93″, E 13° 46′ 16.57″	2011	150	6	10	16
Raná Hrádek (airfield)	N 50° 24′ 13.99″, E 13° 45′ 6.99″	2011	320	6	9	15
Mohelno (National Conservation Area Mohelenská hadcová step)	N 49° 6′ 36.29″, E 16° 11′ 4.52″	2011	75	6	7	13
Vyškov (airfield)	N 49° 18′ 0.99″, E 17° 1′ 30.99″	2015	500	5	14	19
Velká Dobrá (airfield)	N 50° 6′ 45.99″, E 14° 5′ 22.99″	2017	230	9	10	19
Prague Zoo	N 50° 7′ 16.09″, E 14° 24′ 9.31″	2013	70–80	5	9	14
		2015	60–70	4	6	10

The number of recorded individuals and estimated total abundance for the year when the data collection was conducted (Jan Matějů, unpublished data) is provided for each colony. The individuals from the Prague Zoo colony were microchipped (see Schneiderová et al. 2015), ensuring that different individuals were recorded in 2013 and 2015.

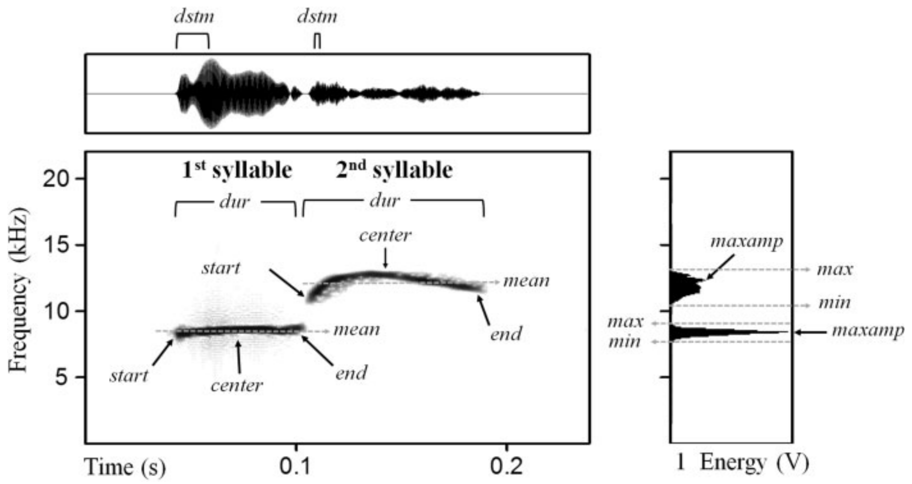

Figure 2. Spectrogram (left, below), power spectrum (right) and waveform (left, above) of an alarm call of the European ground squirrel showing the acoustic parameters measured from the fundamental frequency band of each alarm call syllable; *dur*, the duration of the syllable; *dstm*, distance from the start to the maximum amplitude of the syllable; *start*, starting frequency of the syllable; *end*, ending frequency of the syllable; *center*, center frequency of the syllable; *maxamp*, frequency with the maximum amplitude of the syllable; *mean*, mean frequency of the syllable; *min*, minimum frequency of the syllable; *max*, maximum frequency of the syllable. The parameter expressing frequency modulation of each syllable (*std*) is not shown. Spectrogram settings: sampling rate 44.1 kHz, Hamming window, FFT length 512 points, frame 50%, and overlap 93.75%.

For each syllable, we further calculated 2 acoustic parameters: the localization of the maximum amplitude within the duration of the syllable as disttomax/dur (*peakloc*) and frequency range of the syllable as maximum–minimum frequency (*fr*). In total, we measured and calculated 12 acoustic parameters for each of 2 syllables.

Statistical analysis

The measured values were averaged for each ground squirrel to acquire only a single value for each variable per individual. For the following statistical analyses, we selected 9 acoustic parameters from each of the 2 syllables (*dur*, *peakloc*, *start*, *center*, *end*, *maxamp*, *mean*, *fr*, and *std*), which means that 18 parameters in total were used. The acoustic parameters *dstm*, *min*, and *max* were not used in the statistical analysis as they were used to calculate other acoustic parameters (*peakloc* and *fr*) only. To distinguish the acoustic parameters measured and calculated from the 2 different syllables, those from the first syllable are further indicated by 1 and those from the second syllable are indicated by 2. The Shapiro–Wilk normality test did not show a normal distribution ($P < 0.05$) for some acoustic parameters in 2 datasets from the seminatural colony (the dataset for 2013: *fr1*, *std1*, *peakloc2*, *std2*; and the dataset for 2015: *peakloc1*, *end1*, *center1*, *mean1*). The sex ratio was almost the same for both datasets (1:1.8 for 2013 and 1:1.5 for 2015). Therefore, we did not control for sex and used the Mann–Whitney U test to compare whether there were differences in any of the measured acoustic parameters between the years 2013 and 2015 in the seminatural colony from the Prague Zoo. We performed this test because previous authors demonstrated that the acoustic structure of the alarm calls of some ground-dwelling sciurids can change over time or according to annual changes in habitats in some populations (Perla and Slobodchikoff 2002; Eiler and Banack 2004). Our results showed that there are no such differences in any of the parameters (Table 2); hence, we pooled both datasets and used them together with the datasets from the natural colonies in the following statistical analyses. Descriptive statistics were calculated for each colony (and for both the years 2013 and 2015 in the case of the seminatural colony from the Prague Zoo), and each acoustic parameter and the results are given as the means ± SD.

Because some of the measured acoustic parameters were highly correlated, the data were log-transformed, and we used principal component analysis (PCA) to extract and select several uncorrelated principal components that were used in further statistical analyses. The Shapiro–Wilk normality test was applied to evaluate whether the principal component scores meet the assumption of a normal distribution. We used a general linear model (GLM) to investigate the effect of the colony, sex, and the interaction of the colony and sex on each of the selected principal component scores. The results of the Shapiro–Wilk normality test and GLM were considered significant if $P < 0.05$.

The standard DFA and a leave-one-out cross-validation method were applied to estimate the percentage of the classification of individuals' alarm calls to its colony of origin. In the leave-one-out cross-validation, the classification function is determined by using all but one observation, and this function is then used to predict the group membership of the deleted observation. This process is repeated for each observation so that each observation is classified by a function determined by the others (Huberty and Olejnik 2005). The performance of the classification was evaluated by 2 measures: the percentage of correct classifications, that is, the percentage of individuals from an actual locality correctly classified *a posteriori* into it, and precision of classification, that is, the percentage of individuals correctly classified *a posteriori* into a given colony from all individuals classified into it by DFA (Olson and Delen 2008).

The hierarchical cluster analysis (CLU) with Euclidean distances among colonies based on the colony average values for each principal component was applied to show the acoustic similarities between the natural colonies covered in this study (Romesburg 2004). The analysis was based on the average linkage between-groups (unweighted pair group method with arithmetic mean, UPGMA) method for which we reached the highest value of the cophenetic correlation coefficient ($c = 0.83$). Finally, the correlation between the geographical and acoustic distances of the natural colonies was evaluated with a 1-tailed permutation Mantel test (100,000

Table 2. Descriptive statistics (means ± SD) of the measured acoustic parameters for the 5 natural colonies and for the artificial seminatural colony located at the Prague Zoo

	Acoustic parameter	Raná	Raná Hrádek	Velká Dobrá	Vyškov	Mohelno	Prague Zoo	Prague Zoo 2013	Prague Zoo 2015	Mann–Whitney U Test	
		$n = 16$	$n = 15$	$n = 19$	$n = 19$	$n = 13$	$n = 24$	$n = 14$	$n = 10$	W	*P*
1st syllable	Dur (ms)	69.4 ± 9.7	62.6 ± 7.9	52.8 ± 12.0	70.0 ± 11.8	62.6 ± 9.2	62.6 ± 11.3	60.3 ± 12.6	65.7 ± 8.7	54	0.37
	Peakloc (%)	19.1 ± 6.0	27.0 ± 8.7	16.1 ± 10.3	25.8 ± 11.3	24.5 ± 9.2	30.9 ± 11.5	33.7 ± 10.6	27.1 ± 12.1	98	0.11
	Start *f0* (kHz)	8.3 ± 0.6	8.4 ± 0.6	7.7 ± 0.5	9.0 ± 0.5	7.4 ± 0.4	8.3 ± 0.7	8.2 ± 0.8	8.5 ± 0.5	53	0.34
	End *f0* (kHz)	8.4 ± 0.5	8.4 ± 0.4	7.6 ± 0.5	9.0 ± 0.4	7.5 ± 0.5	8.3 ± 0.6	8.1 ± 0.7	8.6 ± 0.4	46	0.17
	Center *f0* (kHz)	8.5 ± 0.5	8.6 ± 0.4	7.7 ± 0.5	9.2 ± 0.4	7.7 ± 0.5	8.5 ± 0.6	8.3 ± 0.7	8.7 ± 0.5	49	0.24
	Maxamp *f0* (kHz)	8.4 ± 0.5	8.5 ± 0.5	7.7 ± 0.5	9.1 ± 0.5	7.6 ± 0.4	8.4 ± 0.6	8.3 ± 0.7	8.7 ± 0.5	52	0.31
	Mean *f0* (kHz)	8.5 ± 0.5	8.5 ± 0.4	7.7 ± 0.5	9.1 ± 0.4	7.6 ± 0.5	8.4 ± 0.6	8.2 ± 0.7	8.7 ± 0.4	51	0.29
	Fr *f0* (kHz)	0.8 ± 0.2	0.8 ± 0.01	0.7 ± 0.06	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	56	0.43
	Std *f0* (%)	1.1 ± 0.5	1.6 ± 0.8	1.1 ± 1.1	1.2 ± 0.6	1.6 ± 0.5	1.5 ± 0.9	1.5 ± 0.9	1.6 ± 0.8	69	0.98
2nd syllable	Dur (ms)	50.7 ± 18.5	51.3 ± 17.1	43.4 ± 16.9	52.0 ± 17.4	60.8 ± 13.8	48.0 ± 17.1	44.0 ± 16.1	53.6 ± 17.6	48	0.21
	Peakloc (%)	21.7 ± 10.8	33.1 ± 12.2	33.3 ± 11.8	31.9 ± 11.3	25.7 ± 7.4	31.6 ± 10.8	29.4 ± 11.2	34.6 ± 9.9	45	0.15
	Start *f0* (kHz)	12.0 ± 2.0	11.7 ± 1.0	11.0 ± 1.2	12.8 ± 1.2	10.5 ± 0.9	11.2 ± 1.5	11.2 ± 1.5	11.2 ± 1.5	65	0.8
	End *f0* (kHz)	12.6 ± 1.6	11.9 ± 1.3	11.9 ± 0.9	12.4 ± 1.4	10.4 ± 1.0	11.4 ± 1.4	11.5 ± 1.3	11.2 ± 1.6	77	0.71
	Center *f0* (kHz)	13.5 ± 1.4	12.6 ± 1.4	12.1 ± 0.9	13.1 ± 1.2	10.9 ± 0.8	11.9 ± 1.3	11.9 ± 1.3	12.0 ± 1.5	69	0.98
	Maxamp *f0* (kHz)	12.9 ± 1.4	12.4 ± 1.3	11.9 ± 0.9	13.1 ± 1.1	10.7 ± 0.9	11.7 ± 1.4	11.7 ± 1.4	11.8 ± 1.5	64	0.75
	Mean *f0* (kHz)	12.9 ± 1.4	12.3 ± 1.2	11.8 ± 0.8	12.9 ± 1.1	10.7 ± 0.8	11.6 ± 1.3	11.6 ± 1.3	11.6 ± 1.4	68	0.93
	Fr *f0* (kHz)	2.5 ± 1.3	1.7 ± 0.7	1.8 ± 1.2	1.6 ± 0.5	1.2 ± 0.4	1.6 ± 0.6	1.4 ± 0.5	1.8 ± 0.7	47	0.19
	Std *f0* (%)	10.1 ± 8.5	6.5 ± 5.0	4.8 ± 4.9	4.2 ± 3.0	2.5 ± 2.0	6.4 ± 5.4	5.9 ± 5.8	7.0 ± 5.0	57	0.47

The descriptive statistics are given separately in gray for each year when the data were collected at the zoo. The results of the Mann–Whitney U test are given in gray for comparison with the calls collected at the Prague Zoo in 2013 and 2015; n = number of animals.

permutations). All data and statistical analyses were performed in R, version 3.4.1 (R Core Team 2013), and the packages factoextra, MASS, cluster and ape were used.

Results

Descriptive statistics of acoustic parameters of alarm calls for each colony are provided in Table 2. The alarm calls of ground squirrels from different colonies were mostly distinguished by the frequency parameters of both syllables. For example, European ground squirrels from the Velká Dobrá and Mohelno colonies had, on average, a relatively low mean fundamental frequency of the first syllable, 7.7 and 7.6 kHz, respectively, whereas animals from the Vyškov colony had a relatively high mean fundamental frequency of the first syllable, which was 9.1 kHz on average (see also Figure 3). PCA extracted 5 principal components with eigenvalues >1, which accounted for more than 87% of the variability in our data. The detailed eigenvalues, cumulative explained variance given in percentages and factor loadings for each principal component are given in Table 3.

The Shapiro–Wilk normality test revealed that scores at all 5 principal components met the assumption of a normal distribution ($P > 0.05$). The GLM showed that the colony had a significant effect on all principal component scores, with the exception of the 4th principal component (Table 3). The sex and the interaction of the colony and sex had no significant effect on any of the principal components.

Because the GLM revealed that sex does not have any significant effect on the principal component scores, males and females were pooled together for the purpose of the subsequent analyses. The DFA with leave-one-out cross-validation procedure revealed that 55.7% of individuals were classified to the correct colony based on the acoustic structure of their alarm calls. Table 4 provides detailed results of the classification success for each colony, which ranged from 38% to 74%. The colonies also differ in the precision of classification, ranging from 35% to 78%. The percentage of the colony separation achieved by the first 2 discriminant functions (LD1 and LD2) was almost 91% (57% for LD1 and 34% for LD2), with LD1 being highly correlated with PC1 ($r = -0.54$) and LD2 being highly correlated with PC3 ($r = 0.52$).

The dendrogram generated by the CLU reflecting the relationships of the natural colonies based on the acoustic structure of the alarm calls emitted by local ground squirrels revealed 2 clearly separated clusters, with the Raná, Raná Hrádek, and Vyškov colonies forming the first cluster and the Velká Dobrá and Mohelno colonies forming the second cluster (Figure 4).

The shortest geographic as well as acoustic distance were found between the colonies in Raná and Raná Hrádek. The largest geographic distance was found between the colonies in Raná Hrádek and Vyškov, whereas the largest acoustic distance was found between the colonies in Vyškov and Mohelno (Table 5). The Mantel test did not reveal a significant correlation between the geographical and acoustic distances ($P = 0.55$).

Discussion

The results of this study show that there are hardly any differences in the acoustic structure of the alarm calls of male and female European ground squirrels. This is consistent with a previous study showing that sex-related differences are also absent in a relatively less social Eurasian ground squirrel, the speckled ground squirrel *S. suslicus* (Matrosova et al. 2011). In more social ground-dwelling

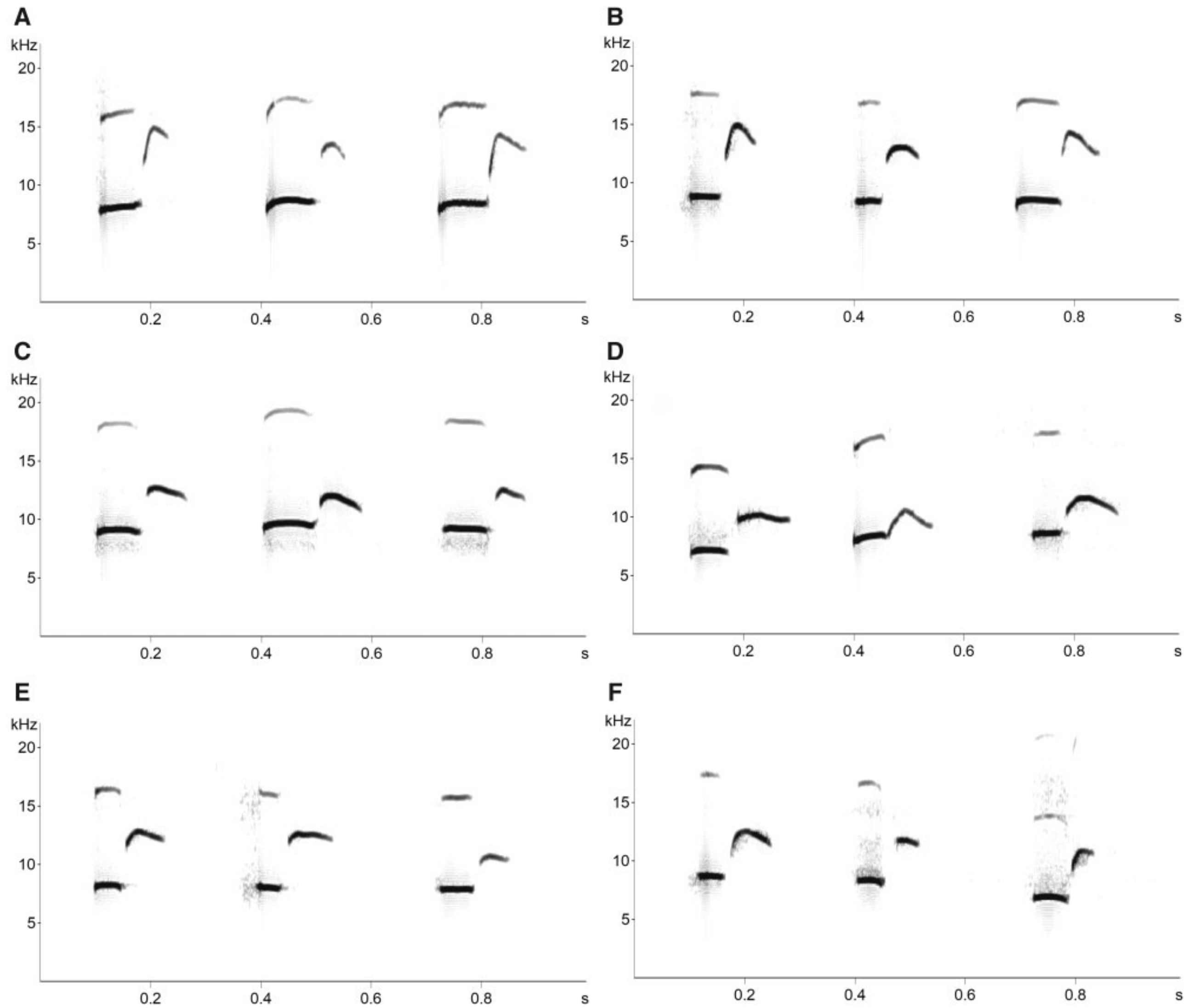

Figure 3. Representative spectrograms of an alarm call of 3 individuals (from the left, 2 females and 1 male) from each study site: (**A**) Raná, (**B**) Raná Hrádek, (**C**) Vyškov, (**D**) Mohelno, (**E**) Velká Dobrá, and (**F**) Prague Zoo.

sciurids, such as the yellow ground squirrel *S. fulvus* and yellow-bellied marmot *Marmota flaviventris*, cues related to sex are more prominent, although still remarkably less expressed than cues to identity (Matrosova et al. 2011).

This study demonstrates that highly isolated and genetically differentiated colonies of the European ground squirrel manifest acoustic differentiation. The DFA showed that the studied colonies differed in the degree of acoustic differentiation, with some of them reaching substantially higher classification success than others. The highest degree of discrimination was found in the most isolated colonies, where 54–74% of individuals were classified correctly. The interconnected colonies of Raná and Raná Hrádek had the lowest classification success, with 38–40% of individuals classified correctly. The individuals from the artificial seminatural colony were often classified correctly to this colony (58% classified correctly); however, the precision of the classification was comparatively relatively low: many individuals from other colonies were incorrectly classified into this seminatural colony, which likely corresponds to the different origins of its founders. We stress that different measures for the evaluation of classification are needed for the interpretation of classification success in DFA.

It was beyond the scope of this study to reliably identify which factors contributed to the observed geographic variability. However, it seems unlikely that the remarkable differences in the acoustic structure of the European ground squirrel's alarm calls are associated with differences in habitat structure, as proposed for some other mammalian taxa (e.g., Perla and Slobodchikoff 2002; Le Roux et al. 2002). All the studied colonies can be defined as open habitats without trees and bushes, but detailed information on their management was unknown. Therefore, we could only roughly divide the colonies into 2 types of habitats: an artificial airfield (Velká Dobrá, Vyškov, Raná Hrádek) and a natural steppe (Raná, Mohelno, and the Prague Zoo). The cluster analysis revealed that the artificial airfields and natural steppes did not show acoustic similarity, whereas the colonies with mutual exchange of animals did show acoustic similarity, although these colonies differed in their habitat structure, with Raná being a natural steppe and Raná Hrádek being an artificial airfield.

Table 3. Results of PCA, including the eigenvalues, cumulative variance explained given in percentages and the factor loadings for the 5 principal components with eigenvalues higher than 1

		PC1	PC2	PC3	PC4	PC5
	Eigenvalue	6.7	3.7	2.6	1.6	1.2
	Cumulative variance explained (%)	36.9	57.5	71.6	80.2	86.8
1st syllable	*Dur*	−0.02	**0.32**	−0.06	**0.34**	−0.14
	Peakloc	−0.06	−0.03	0.09	−0.20	**0.75**
	Start	**−0.33**	0.22	0.17	−0.12	0.01
	End	**−0.32**	**0.25**	0.11	−0.05	−0.04
	Center	**−0.32**	**0.27**	0.13	0.00	0.00
	Maxamp	**−0.32**	**0.26**	0.15	−0.05	0.04
	Mean	**−0.32**	**0.27**	0.14	−0.03	−0.01
	Fr	0.05	**0.27**	−0.14	**0.56**	−0.12
	Std	0.13	0.16	−0.05	**0.49**	**0.33**
2nd syllable	*Dur*	0.04	**0.28**	**−0.29**	−0.16	0.17
	Peakloc	−0.08	−0.13	**0.28**	−0.11	**−0.48**
	Start	**−0.30**	**−0.25**	0.07	0.21	0.12
	End	**−0.27**	**−0.30**	−0.18	0.15	−0.04
	Center	**−0.30**	−0.21	**−0.29**	0.09	0.02
	Maxamp	**−0.32**	**−0.24**	−0.18	0.10	0.03
	Mean	**−0.31**	**−0.25**	−0.19	0.13	0.03
	Fr	−0.06	0.17	**−0.53**	−0.22	−0.10
	Std	−0.04	0.18	**−0.48**	**−0.27**	−0.12
GLM	Colony	$F = 21.79$	$F = 6.45$	$F = 5.23$	$F = 1.59$	$F = 4.93$
	($df = 5, 100$)	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$P = 0.17$	$\mathbf{P < 0.001}$
	Sex	$F = 0.94$	$F = 0.80$	$F = 0.37$	$F = 1.59$	$F = 0.43$
	($df = 1, 99$)	$P = 0.34$	$P = 0.37$	$P = 0.55$	$P = 0.21$	$P = 0.51$
	Colony/sex interaction	$F = 0.66$	$F = 0.90$	$F = 0.63$	$F = 1.02$	$F = 0.96$
	($df = 5, 94$)	$P = 0.66$	$P = 0.49$	$P = 0.68$	$P = 0.41$	$P = 0.45$

The values in bold represent contributions of acoustic parameters that are above the expected value if the contributions were uniform.
GLM results on the effects of sex and colony on the 5 principal components, the *F*-statistics and *P*-values are also provided and are in bold when $P < 0.05$.

Table 4. *A posteriori* classification of the alarm calls of the European ground squirrels among the 6 studied colonies by the cross-validated DFA

		Predicted colony						
Actual colony	*n*	Raná	Raná Hrádek	Vyškov	Velká Dobrá	Mohelno	Prague Zoo	Correct classification (%)
Raná	16	6*	3	3	1	0	3	**37.5**
Raná Hrádek	15	5	6*	1	1	0	2	**40.0**
Vyškov	19	2	1	12*	1	0	3	**63.2**
Velká Dobrá	19	0	3	0	14*	0	2	**73.7**
Mohelno	13	0	0	0	1	7*	5	**53.8**
Prague Zoo	24	4	1	2	1	2	14*	**58.3**
Precision of classification (%)		**35.0**	**42.9**	**66.7**	**73.7**	**77.8**	**48.3**	**55.7**

n = number of individuals; *Indicates the number of correctly classified individuals

The results of this study support the conclusions that the observed geographic variability in the alarm calls of the European ground squirrel is probably a result of genetic drift or vocal learning. However, the actual contribution of these 2 phenomena cannot be easily distinguished. Under genetic drift, the divergence should increase with a geographic or genetic distance (Wilkins et al. 2013). Unlike in the speckled ground squirrel (Matrosova et al. 2016), our results did not support a correlation between the geographical and acoustic distances of the studied colonies. However, a major issue limiting further conclusions is the unclear origin of one of the natural colonies located in the Velká Dobrá airfield. Until the genetic data confirm the origin of the European ground squirrels from this colony, any attempts to investigate correlations between the geographical and acoustic distances of the studied colonies will fail. Regardless, a previous study based on genetic data determined that the Vyškov colony is more similar to the more distant colony, Raná Hrádek, than to geographically closer colonies (Hulová and Sedláček 2008), which is the same pattern shown by our study based on the acoustic data. Therefore, an association between the acoustic and genetic distance cannot be excluded and should be appropriately tested in the future.

Vocal learning significantly contributes to the variability of calls in some mammalian species (Janik and Slater 1997). To what extent this may apply to the alarm calls of the European ground squirrel remains unclear. Species specificity of the alarm calls of ground-

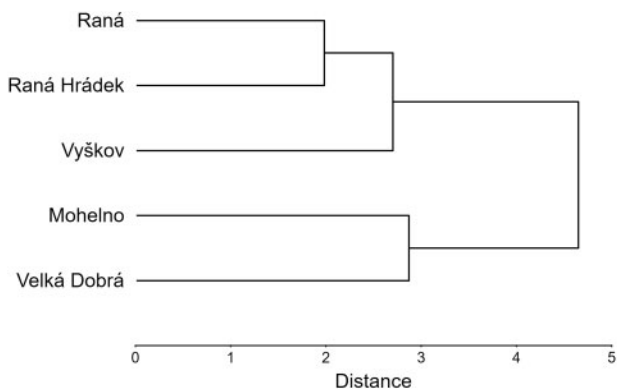

Figure 4. Dendrogram showing relationships of the studied natural colonies based on acoustic structure of the alarm calls of European ground squirrels. The dendrogram was created by hierarchical cluster analysis using the UPGMA method based on Euclidean distances. The seminatural artificially established colony in the Prague Zoo was not included in the cluster analysis.

Table 5. Geographical distance (in kilometer, above the diagonal) and acoustic distance (in Euclidean distances, below the diagonal) among the 5 studied natural colonies

	Raná	Raná Hrádek	Vyškov	Velká Dobrá	Mohelno
Raná		1.40	263.7	39.8	225.5
Raná Hrádek	1.98		264.8	40.30	226.3
Vyškov	2.40	3.00		229.6	64.3
Velká Dobrá	3.65	3.34	4.98		187.6
Mohelno	4.71	3.27	5.95	2.87	

dwelling sciurids is probably innate and genetically predetermined, as hybridization of different species results in an intermediate acoustic structure of alarm calls (Koeppl et al. 1978; Nikol'skii et al. 1984). Moreover, it was shown experimentally that isolated ground squirrel juveniles developed alarm calls with the same basic acoustic structure as adults (Matocha 1975). Interspecies cross-fostering experiments also confirmed the genetically predetermined basic acoustic structure of alarm calls. However, subtle differences in the alarm calls of juveniles reared by their mother and by different species indicated that some learning is possibly involved in alarm call development (Matocha 1975).

The results of this study may have implications for the European ground squirrel's action plan and specifically for the reintroductions recently occurring in the Czech Republic and elsewhere (Matějů et al. 2010b). For example, Martins et al. (2018) highlighted the value of the acoustic analysis of flight calls of the cactus conure *Eupsittula cactorum* for revealing the origin of confiscated birds and their later proper reintroductions. This study indicates that in newly and artificially established colonies, European ground squirrels of various origin can be exposed to unfamiliar social environments and specifically to unfamiliar alarm calls. Previous research demonstrated that ground-dwelling sciurids can distinguish between the alarm calls of neighbors and non-neighbors, being more susceptible to the alarm calls of the neighboring individuals (Hare 1998; Hare and Atkins 2001; Blumstein et al. 2004). However, no experiments investigating how these rodents respond to the alarm calls of individuals from different colonies have been conducted so far. Lower responsiveness to unfamiliar alarm calls could cause higher predation costs (Sherman 1977), whereas higher responsiveness could be costly in terms of time and energy (Arenz and Leger 2000). For the animals, it is critical to find an optimal solution to the trade-off between predation risk and resource acquisition (Pollard 2011), and the capability of ground squirrels to find this trade-off might be decreased in newly established artificial colonies. This could result in the decreased viability of such colonies given the importance of alarm calls in their antipredatory strategy. However, previous studies focused on the ontogeny of alarm call responses (Mateo 1996; Mateo and Holmes 1999), on the changes in responses to unreliable callers (Hare and Atkins 2001) or to the novel sounds or alarm calls of other species (Shriner 1998, 1999) demonstrated that learning takes place in ground squirrels in response to alarm calls. Therefore, it is probable that the European ground squirrels can become familiar with their new social environment, including unfamiliar alarm calls, if they are given appropriate conditions and enough time.

Acknowledgments

We thank Magda Starcová, Petra Caltová, Kristýna Nováková, Jitka Větrovcová, Jitka Uhlíková, Petra Schnitzerová, Jan Matějů, and Vladimír Vohralík for their help in the field. We thank the aero clubs in Vyškov, Raná Hrádek, and Velká Dobrá, and the Prague Zoo for their cooperation with this study. During our work, we adhered to the "Guidelines for the treatment of animals in behavioral research and teaching" (2006, *Anim Behav* 71: 245–253). The study was conducted under the Action Plan for the European ground squirrel in the Czech Republic and under the permission of Český kras Protected landscape area no. 00225/CK/E/06 and Regional Authority of the Central Bohemian Region no. 033290/2017/KUSK.

Funding

The study was partially supported by the Ministry of the Environment of the Czech Republic (project no. SP/2d4/61/08).

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