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RESEARCH ARTICLE

Differences in Alarm Calls of Juvenile and Adult European Ground Squirrels (*Spermophilus citellus*): Findings on Permanently Marked Animals From a Semi-Natural Enclosure

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The European ground squirrel (*Spermophilus citellus*) emits alarm calls that warn conspecifics of potential danger. Although it has been observed that inexperienced juveniles of this species emit alarm calls that sound similar to those of adults, studies focusing on juvenile alarm calls are lacking. We analyzed the acoustic structure of alarm calls emitted by six permanently marked European ground squirrels living in a semi-natural enclosure when they were juveniles and after 1 year as adults. We found that the acoustic structure of the juvenile alarm calls was significantly different from those of adults and that the alarm calls underwent nearly the same changes in all studied individuals. All juveniles emitted alarm calls consisting of one element with almost constant frequency, but their alarm calls included a second frequency-modulated element after their first hibernation as adults. Our data show that the duration of the first element is significantly shorter in adults than in juveniles. Additionally, the frequency of the first element is significantly higher in adults than in juveniles. Similar to previous findings in other Palearctic ground squirrel species, our data are inconsistent with the assumption that juvenile mammals emit vocalizations with higher fundamental frequencies than adults. However, our results do not support the previously suggested hypothesis that juvenile ground squirrels conceal information regarding their age in their alarm calls because we found significant differences in alarm calls of juveniles and adults. Zoo Biol. 34:503–512, 2015.

Keywords: age-related differences; bioacoustics; Sciuridae; semi-captive population; vocalization

INTRODUCTION

Ground-dwelling sciurids are diurnal and primarily colonially living small or middle-sized rodents preyed on by numerous predators, including terrestrial carnivores, raptors, and snakes [Murie and Michener, 1984; Thorington et al., 2012]. An essential part of their antipredator behavior is to warn their kin of a potential threat via the emission of alarm calls [Owings and Hennessy, 1984; Blumstein, 2007]. The alarm call increases the vigilance of the receiver, thereby enhancing the early detection of the predator and increasing the likelihood of a successful escape [Weary and Kramer, 1995]. Moreover,

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ground-dwelling sciurids receiving alarm calls can adjust their responses precisely because in these species, the acoustic structure of the alarm calls conveys a large amount of information, including details on imminent threats or their senders [e.g., Owings and Hennessy, 1984; Blumstein and Arnold, 1995; Warkentin et al., 2001; Blumstein and Munos, 2005; Blumstein, 2007; Slobodchikoff et al., 2009; Matrosova et al., 2011].

Although inexperienced juveniles can produce the alarm calls shortly after their emergence, their acoustic structure can differ slightly from that of adults [Blumstein and Munos, 2005; Nikol'skii, 2007; Matrosova et al., 2011]. Adult California ground squirrels (*Otospermophilus beecheyi*) discriminate between juvenile and adult alarm calls and respond with less urgency to the calls of juveniles [Hanson and Coss, 2001]. Blumstein and Daniel [2004] demonstrated that yellow-bellied marmots (*Marmota flaviventris*) also discriminate between alarm calls of these two age classes, but that the marmots are more responsive to the alarm calls of juveniles. However, Swan and Hare [2008] showed no differences in the responses of Richardson's ground squirrels (*Urocitellus richardsonii*) to adult and juvenile alarm calls, which have a similar acoustic structure. Recent studies indicate that juveniles of some Palearctic ground squirrels may conceal information regarding their age in their alarm calls, mainly by producing similar fundamental frequencies to those of adults. These indistinguishable fundamental frequencies are manifested despite significant differences in their body size [Matrosova et al., 2007; Volodina et al., 2010].

The fundamental frequency of vocalizations is determined by the vocal apparatus, specifically by the larynx in mammals. It is generally accepted that larger vocal folds in larger species or individuals can produce lower fundamental frequencies [Fitch and Hauser, 2002]. Therefore, larger adults should be able to produce lower fundamental frequencies than smaller juveniles, and this has been observed in some mammalian species [Elowson et al., 1992; Blumstein and Munos, 2005; Hartwig, 2005; Nikol'skii, 2007]. However, this is not true for several small mammals [Volodin et al., 2014], including the speckled ground squirrel (*Spermophilus suslicus*) and the yellow ground squirrel (*S. fulvus*) [Matrosova et al., 2007; Volodina et al., 2010]. The "vocal mimicry" hypothesis advanced by Matrosova et al. [2007] assumes that juveniles, with their more flexible larynges, lower the pitch of their alarm calls to avoid age-dependent risk. Juvenile mammals may suffer higher predator pressure than adults [Donázar and Ceballos, 1989; Barja, 2009], and non-parental infanticide has also been reported to be a major source of juvenile mortality in some ground-dwelling sciurids [Ebensperger and Blumstein, 2007].

The European ground squirrel (*Spermophilus citellus*), also known as the European souslik, is currently listed by the IUCN (International Union for Conservation of Nature) as vulnerable in Europe [Coroiu et al., 2008] and as critically endangered in the Czech Republic [Anděra and Červený, 2003]. Several conservation measures were recently adopted to prevent the potential extinction of this rodent species in the Czech Republic. Establishing semi-captive colonies that serve as a source of animals for later reintroductions was one such measure and was implemented by some Czech zoos [Matějů et al., 2010]. Similar to many other ground-dwelling sciurids, this species is an obligatory hibernating rodent that usually immerges into hibernation between August and September and emerges from hibernation between March and the end of April [Grulich, 1960; Matějů, 2008]. Juveniles are born during May and leave their maternal burrow for the first time at the age of 28 days, reaching a body weight from 40 to 50 g. Juveniles with body weights from 90 to 100 g begin to stray farther from their maternal burrow and finally completely leave the burrow [Grulich, 1960].

The European ground squirrel inhabits short-steppes and similar artificial habitats. In these environments, individuals can scan their surroundings to detect predators and emit alarm calls on time. European ground squirrels also emit alarm calls in the presence of humans, and typically utter them repeatedly in longer series lasting the duration of the alarm-evoking stimulus [Schneiderová, 2012]. The alarm call of the European ground squirrel comprises two elements, of which the first element has limited frequency modulation whereas the second element is more frequency modulated [Koshev and Pandourski, 2008; Schneiderová and Policht, 2012]. Alarm calls containing only one of these two elements were also recorded from some individuals [Koshev and Pandourski, 2008; Schneiderová and Policht, 2012], and the possible adaptive significance of such structural variability has not yet been clarified [Schneiderová, 2012]. Figure 1 shows a representative spectrogram of several alarm calls typical for the European ground squirrel. Similar to that in other ground-dwelling sciurids [Nikol'skii and Suchanova, 1994; McCowan and Hooper, 2002; Blumstein and Munos, 2005; Volodin, 2005; Pollard et al., 2010; Matrosova et al., 2011; Pollard and Blumstein, 2011], the alarm call of this species manifests with high short-term individuality, such that calls recorded from one individual are highly similar within one recording session but differ significantly from calls produced by different individuals [Schneiderová and Policht, 2010]. It was previously demonstrated that the first and the second element do not differ in this respect [Schneiderová, 2012]. Studies examining the acoustic structure of the European ground squirrels in the long term with permanently marked individuals are not available.

Although juvenile European ground squirrels emit alarm calls that sound similar to those of adults shortly after their first emergence from the maternal burrow [Schneiderová, personal observervation], there are no studies of juvenile alarm call of this species. Therefore, the objective of our study was to analyze the acoustic structure of alarm calls produced by juveniles before their dispersal, when they are younger than 2 months, and compare it to the acoustic structure of their alarm calls emitted at 1 year, after the first hibernation.

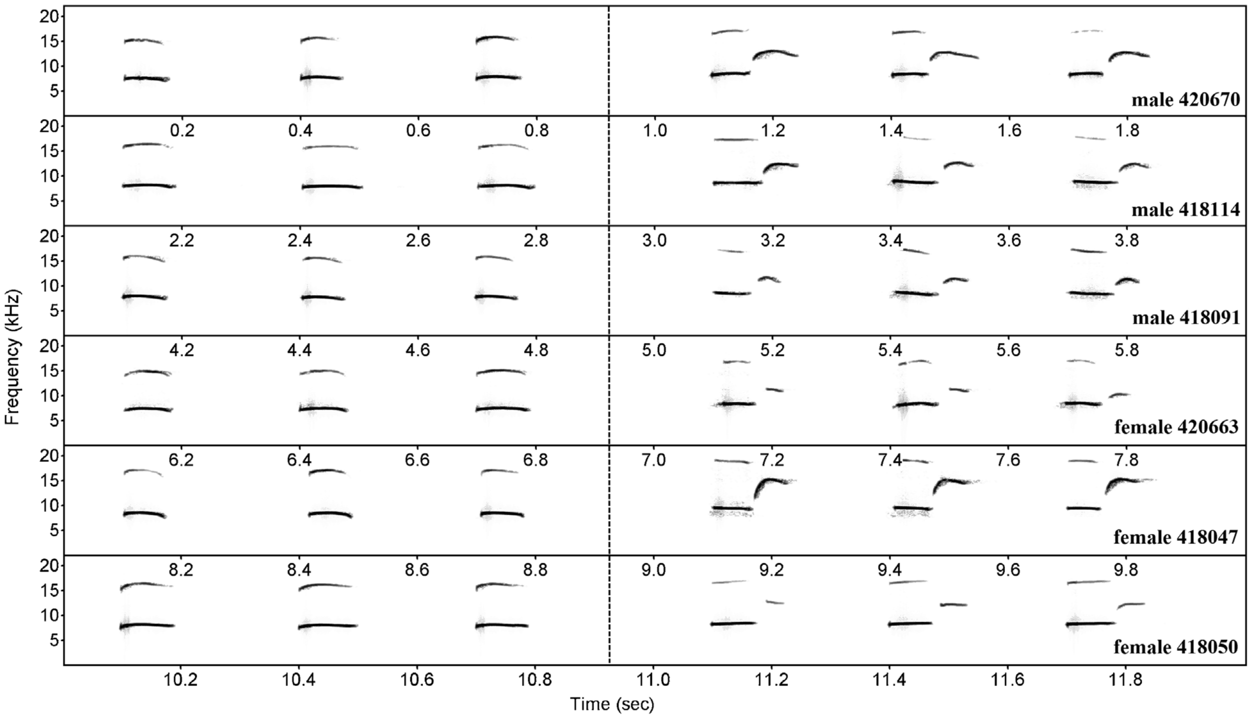

Fig. 1. Representative spectrograms of three alarm calls comprising the only first element emitted by six juvenile European ground squirrels (left) and three alarm calls comprising the first and second element emitted by the same individuals after 1 year, after they became adults (right).

METHODS

Study Site and Animals

The study was conducted in two consecutive years, 2012 and 2013, in a semi-natural outdoor enclosure located at the Prague Zoo (N 50°07′16.8″, E 14°24′10.9″), 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. The location of the enclosure is formed by a natural habitat inhabited by European ground squirrels until the 1960s [Vohralík and Řeháková, 1985]. The enclosure was built in 2006 as a measure proposed in a prepared Action plan for the European ground squirrel in the Czech Republic. The primary objective of this measure was to establish a stable colony to serve as a source of animals for later reintroductions.

The ca. 140 m^2 sized enclosure was constructed from a strong wire mesh that extends 2 m below ground and is equipped with a small wooden shelter. Seventy-three ground squirrels marked with microchips (Planet ID, Essen, Germany), with 33 males and 40 females originating from four natural populations located in the Czech Republic (Prague Letňany, airfield; Raná, National Nature Reserve; Raná Hrádek, airfield; and Bezděčín, airfield), were gradually introduced to the enclosure from 2006 to 2011. We noted successful breeding of the introduced ground squirrels inside the enclosure in 2008, 2009, 2010, 2012, and 2013. According to our estimates, the enclosure was inhabited by 50 individuals at the end of the ground squirrel active season in 2012 and by 60 individuals at the end of the active season in 2013. The population density in the enclosure was many times higher than the mean population density of 15.1 ind./ha (min 2.1 and max. 58.7 ind./ha) in natural colonies in the Czech Republic observed between 2002 and 2008 [Matějů et al., 2008]. During their active season, from March to September, the ground squirrels in the enclosure were provided with various vegetables, such as carrot, beet, and celery, apples, and seeds *ad libitum*.

Our study animals were six European ground squirrels that were observed during one recording session per individual in 2012 as juveniles, 2–3 weeks after their first emergence from the natal burrow. Their body weight ranged from 65 to 100 g (average 85.8 g). These ground squirrels were observed again during one recording session per individual in 2013 as adults, with body weight ranging from 200 to 285 g (average 245.8 g). These ground squirrels included three males (ID 420670, 418114, and 418091) and three females (ID 420663, 418047, and 418050).

Data Collection

During our study, we followed the “Guidelines for the Treatment of Animals in Behavioural Research and Teaching” [ASAB/ABS, 2012]. Recordings of ground squirrel alarm calls were collected from 9 AM to 6 PM on the 28th and 29th of June in 2012, on the 28th and 30th of

June and on the 2nd, 5th and 6th of July in 2013. In 2012, we recorded the alarm calls of 17 juveniles (nine males and eight females), and these juveniles probably represented the majority of juveniles born that year in the enclosure. We live-trapped and recorded only six of these individuals the next year. During the second day of our study in 2013, 72% of all trapping were re-captures of the same individuals previously live-trapped that year. Moreover, we did not observe captures of any new previously marked individual at the end of the study in 2013. We could not ascertain why the number of re-captured and repeatedly recorded individuals was limited, but it is possible that a large number of individuals did not survive their first hibernation. High mortality rates (>80%) in juveniles during hibernation were observed in a wild population with low predation pressure [Matějů, 2008]. In addition, our recapture success of 35% exceeded the recapture success of 22% for the speckled ground squirrel (Tchabovsky, personal communication) and 14% for the yellow ground squirrel (Vasilieva, personal communication).

Ground squirrels were live-trapped with traps constructed from wire mesh and baited with apple or carrot. In these traps, the ground squirrels emitted alarm calls spontaneously toward a researcher (IS) with recording equipment standing approximately 3 m away. This procedure is generally accepted for ground-dwelling sciurids [see, e.g., Hanson and Coss, 2001; Blumstein and Daniel, 2004; Blumstein and Munos, 2005; Matrosova et al., 2009] because the calls collected from live-trapped individuals are indistinguishable by human ear from those produced by free-ranging individuals. In this study, the alarm calls collected from live-trapped animals were also spectrographically similar to those produced by free-living European ground squirrels [see Schneiderová and Policht, 2010]. It was previously demonstrated in the yellow ground squirrel that alarm calls recorded from live-trapped individuals do not significantly differ from those recorded from free-ranging animals [Matrosova et al., 2010].

The solid state recorder Marantz PMD-661 (D & M Professional, Kanagawa, Japan) with internal microphones using a 16-bit recording format with sampling rate of 44.1 kHz and frequency response of 20–20,000 Hz was used as the recording equipment. After the recording, each ground squirrel was removed from the trap, weighed (using a PESOLA scale, ± 5 g, max 600 g, PESOLA AG, Baar, Switzerland) and marked with microchips in 2012, or its microchip number was noted in 2013. After these procedures, each ground squirrel was released into the burrow near where it was live-trapped and recorded.

Acoustic Analysis

We used the Avisoft SASLab Pro software (Specht, Berlin, Germany) for the detailed acoustic analysis. We carefully checked all obtained recordings to evaluate the quality of the recording and the general acoustic structure of the recorded alarm calls. From all recorded juveniles in 2012 and adults in 2013, we selected those that were recorded as both juveniles and adults. A total of six individuals were recorded with a minimum of 10 to a maximum of 33 alarm calls per recording session. For each ground squirrel, we randomly selected 10–15 alarm calls of the highest quality from the first year, when the ground squirrels were juveniles, and approximately the same number of alarm calls from the second year, when the ground squirrels were adults.

The selected alarm calls (163 in total) were visualized and labeled using a waveform and spectrogram with the following settings: Hamming window, FFT length 512, frame size 50%, and overlap 93.75%. We used the "automatic parameter measurements" tool to measure the duration (*duration*) of the entire alarm calls and six acoustic parameters for each element of the calls. These parameters included the duration (*dur*) of each element, location of the maximum amplitude (*peak loc*) within the duration of each element, the starting fundamental frequency (*start f*0) of each element, the ending fundamental frequency (*end f*0) of each element, the mean fundamental frequency (*mean f*0) of each element, and the frequency modulation expressed as a relative standard deviation of the fundamental frequency (*std f*0) of each element. Table 1 provides the summary, descriptions, and units of measurements for all measured acoustic parameters.

TABLE 1. Summary, descriptions, and units of measurement of the acoustic parameters measured from alarm calls of the six studied European ground squirrels

Acoustic parameter	Description and unit
Duration	Duration of the entire alarm call (ms)
Dur	Duration of the element (ms)
Peak loc	Location of the maximum amplitude within the duration (% of element's duration)
Start f_0	Starting frequency of the element (kHz)
End f_0	Ending frequency of the element (kHz)
Mean f_0	Mean fundamental frequency of the element (kHz)
Std f_0	Frequency modulation of the element (%)

The duration was measured for the entire alarm call, whereas the remaining parameters were measured for each element of the alarm calls separately.

Statistical Analysis

We used Statistica 12 (StatSoft, Inc., Tulsa, OK) and Stata 13 (StataCorp LP, College Station, TX) for the statistical analyses. The descriptive statistics for the body weight and each measured acoustic parameter are given as $x \pm$ SD. Because the frequency parameters (*start f0*, *end f0*, and *mean f0*) measured from the first element were highly correlated ($r > 0.9$), we used principal component analysis (PCA) to extract the uncorrelated principal components from all parameters measured from this element and applied varimax rotation to the principal loadings to facilitate their interpretation. In all statistical analyses of the acoustic structure of the first element, we used the principal components rather than the original variables.

The PCA extracted six principal components from all parameters measured from the first element of the alarm calls. The first principal component (PC1) was most highly correlated with the frequency parameters (*start f*0, *end f*0, and *mean f*0), the second principal component (PC2) correlated with the location of the maximum amplitude (*peak loc*), the third principal component (PC3) correlated with the frequency modulation (*std f*0), and the fourth principal component (PC4) correlated with the duration (*dur*) (Table 2). We selected these four principal components with factor loadings >0.8 for subsequent statistical procedures (factor loadings in the remaining two principal components did not exceed the value of 0.3, and the components explained less than two percent of the total variance). We did not follow standard procedures for principal components selection, e.g., the Kaiser–Guttman criterion or the broken stick model [Jackson, 1993]. Instead, we primarily aimed to reduce the number of highly correlated frequency parameters (*starf f*0, *end f*0, and *mean f*0), while maintaining information that would allow us to clearly demonstrate structural changes in other measured acoustic parameters, i.e., duration (*dur*), location of the maximum amplitude (*peak loc*), and frequency modulation (*std f*0).

We used a series of one-way repeated measures ANOVAs to test the influence of age on the acoustic structure of the first element of the alarm calls recorded during the first and second years. The same test was used to assess the influence of age on the duration of the alarm calls (*duration*) and on the body weight of the ground squirrels. We used Pearson correlations to study the relation between body weight and frequency parameters of the first element. We examined this relation using pairwise correlations within each age category; statistical inference concerning correlation significance was based on 10,000 replications of non-parametric bootstrap. In both the repeated measures ANOVAs and the correlation analysis, the values of the acoustic parameters measured from multiple alarm calls of each individual were averaged into a single value.

To determine whether individually distinctive alarm calls of juveniles could be discriminated after 1 year, we performed three discriminant function analyses (DFA) to estimate the percentages of correct classifications of first elements to individuals. In the first DFA, we calculated this estimate for the first-year data, when the individuals were juveniles, and in the second DFA, we calculated this estimate for the second-year data, when the individuals were adults. In the third DFA, we classified the first elements from the second year with the discriminant functions derived from the first elements from the first year. The value of the correct cross-validation procedure is considered a measure of the retained individuality after some time and has been used previously in studies of the time stability of individuality in animal vocalizations [Klenova et al., 2009; Matrosova et al., 2010; Odom et al., 2013]. We used 2×2 chi-square tests to compare the values of the correct classifications calculated by DFA from the first year and second year and the values of the correct classifications calculated by DFA from the first year and by cross-validation.

The use of one-way repeated measures ANOVA and DFA seemed legitimate as the Kolmogorov–Smirnov test showed that the residuals did not depart from normality ($P > 0.05$) for the one-way repeated measures ANOVA and departed from normality ($P < 0.05$) in only one case for the DFA. Furthermore, these statistical procedures are generally robust to departures from normality [Lindman, 1974].

RESULTS

Descriptive statistics of body weight and all measured acoustic parameters are presented in Table 3. The body weight of the ground squirrels was significantly higher for

TABLE 2. Percentages of variability explained by the selected principal components, their factor loadings, and the results of one-way repeated measures ANOVA testing the influence of age on the acoustic structure of the first element of six European ground squirrel alarm calls

Principal component	Variability explained (%)	Acoustic parameter						ANOVA	
		Dur	*Peak loc*	*Start f*0	*End f*0	*Mean f*0	*Std f*0	$F_{1,5}$	*P*
PC1	64.92	−0.34	0.16	**0.87**	**0.89**	**0.93**	−0.33	24.41	<0.01
PC2	14.25	−0.15	**0.97**	0.16	0.13	0.13	−0.10	7.09	<0.05
PC3	11.93	−0.08	0.10	0.26	0.34	0.19	**−0.93**	6.52	*ns*
PC4	7.38	**−0.92**	0.13	0.33	0.21	0.24	−0.08	10.52	<0.05

F, *F*-ratio of ANOVA; *P*, significance level; *ns*, non-significant result. Bold values indicate loadings above 0.8 in magnitude.

TABLE 3. Descriptive statistics for the body weight and acoustic parameters measured from the alarm calls of European ground squirrels

	Ground squirrels ($n = 6$)	
Acoustic parameter	Juveniles	Adults
Body weight (g)	85.8 ± 14.6	245.8 ± 27.5
Duration (ms)	78.2 ± 9.9	122.9 ± 12.9
First element		
Dur (ms)	78.2 ± 9.9	61.4 ± 8.1
Peak loc (%)	22.1 ± 5.5	33.3 ± 6.4
Start f_0 (kHz)	7.7 ± 0.3	8.6 ± 0.5
End f_0 (kHz)	7.6 ± 0.3	8.6 ± 0.4
Mean f_0 (kHz)	7.9 ± 0.3	8.7 ± 0.4
Std f0 (%)	1.6 ± 0.3	1.0 ± 0.002
Second element		
Dur (ms)		46.3 ± 13.8
Peak loc (%)		37.8 ± 7.1
Start f_0 (kHz)		11.5 ± 0.8
End f_0 (kHz)		12.0 ± 1.5
Mean f_0 (kHz)		12.0 ± 1.2
Std f0 (%)		2.3 ± 1.4

n, number of alarm calls.

adults than juveniles ($F_{1,5} = 438.9$, $P < 0.001$). The ground squirrels emitted alarm calls comprising one element with almost constant frequency when they were juveniles and alarm calls comprising two structurally different elements, a first with almost constant frequency and a second more frequency modulated, when they became adults (Fig. 1). Therefore, the duration (*duration*) of the entire alarm call was significantly higher in adults than juveniles ($F_{1,5} = 75.75$, $P < 0.001$; Fig. 2). The exclusive emission of alarm calls containing only the first element was also observed in the remaining juveniles recorded in 2012.

Repeated measures ANOVA indicated that except for frequency modulation, the acoustic structure of the first element changed significantly with age (Table 2), and we found nearly the same trends in these changes for each ground squirrel (Fig. 3). The duration of the first element (*dur,* negatively correlated with PC4) was lower in the adults than juveniles, whereas the frequency parameters (*start* f_0, *end* f_0, *mean* f_0, all positively correlated with PC1) were higher in adults than juveniles. With the exception of one individual (male 420670), the location of the maximum amplitude (*peak loc*, positively correlated with PC2) was higher in adults than juveniles. Although we did not obtain a statistically significant result, the frequency modulation (*std f0*, negatively correlated with PC3) was reduced in adults compared to juveniles.

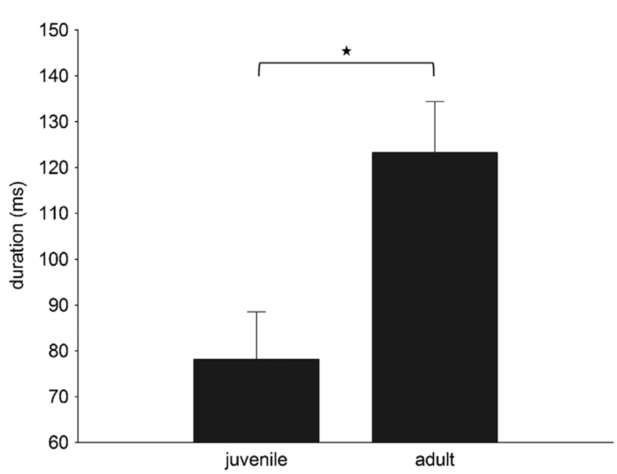

Fig. 2. Average duration of alarm calls emitted by six European ground squirrels as juveniles and after 1 year as adults. The star indicates a significant difference at $P < 0.001$.

There was a significant negative correlation between body weight and PC1 (representing frequency parameters) in juveniles ($r = -0.93$, $P < 0.001$), but no correlation was found in adults ($r = -0.008$, $P = 0.99$). Smaller juveniles emitted alarm calls with higher frequency parameters than larger juveniles (Fig. 4).

DFAs and the cross-validation procedure also showed that the alarm calls of juveniles were significantly changed in structure after 1 year (Table 4). The classification success of 88.8% calculated by DFA for the first-year data did not differ significantly from the classification success of 71.6% calculated by DFA for the second-year data ($\chi^2 = 0.62$, $df = 1$, $P = 0.43$), but cross-validation success of the first-year data discriminations significantly decreased to 24.3% when applied to the second-year data ($\chi^2 = 18.01$, $df = 1$, $P < 0.001$).

DISCUSSION

Our study showed that alarm calls produced by six permanently marked juvenile European ground squirrels living in a semi-natural outdoor enclosure were significantly different from the alarm calls of the same individuals after 1 year. As juveniles, all ground squirrels emitted alarm calls exclusively comprising the first element with a constant frequency only, but their alarm calls included a second frequency-modulated element as adults, after their first hibernation. The exclusive presence of alarm calls containing the first element only was also observed in other juveniles that were recorded when performing this study, which supports the conclusion that such alarm calls are typically emitted by juveniles living in the enclosure. Additionally, our results showed that there were also significant differences between juveniles and adults in the majority of the acoustic parameters measured from the first element.

With their larger lung capacity, larynges, and vocal tracts, adult mammals are able to produce vocalizations of longer duration, lower fundamental frequencies, and lower formants than juveniles [Fitch and Hauser, 2002]. The assumption about the duration is fulfilled by our study because the alarm calls of our adult ground squirrels were significantly longer because they included a second element. Notably, each alarm call, even if it contains both elements, is

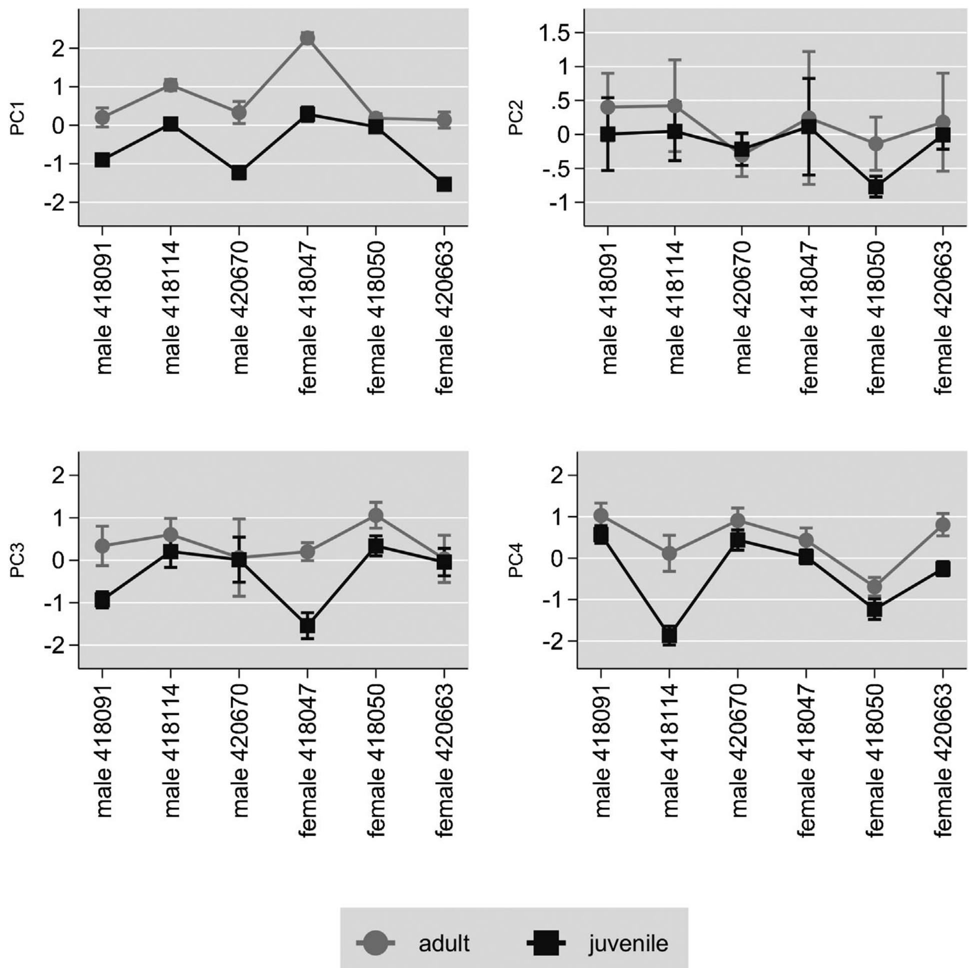

Fig. 3. Changes in the principal components extracted from the six acoustic parameters measured from the first element of alarm calls emitted by six European ground squirrels as juveniles (black line and squares) and 1 year later as adults (gray line and rounds). PC1 represents the frequency parameters *start f*0, *end f*0, and *mean f*0 with high positive loadings for these acoustic parameters (0.87, 0.89, and 0.93, respectively). PC2 represents the location of the maximum amplitude (*peak loc*) with high positive loading (0.97), PC3 is the frequency modulation (*std f*0) with high negative loading (−0.93), and PC4 is the duration (*dur*) with high negative loading (−0.92). The arrangement of the two lines demonstrates that the age-related changes in the acoustic structure of the alarm calls are similar among individuals.

emitted during a single expiration by European ground squirrels, as can be inferred by chest movements of the vocalizing individuals [Schneiderová, personal observation] and by the existence of an extremely short break, essentially indistinguishable by the human ear, between the two elements. In some individuals, alarm calls where the two elements are joined can also be observed [Schneiderová and Policht, 2012]. Significantly longer alarm calls of adults, as compared to juveniles, were also observed for the speckled ground squirrel [Volodina et al., 2010]. Unlike the alarm call of the European ground squirrel, the alarm call of the speckled ground squirrel consist of only one element with almost constant frequency [Nikol'skii, 1979; Volodin, 2005].

Formants are not well expressed in the alarm calls of the European ground squirrel [Volodina et al., 2010], but the fundamental frequency can be easily measured, and the potential effect of an animal's body size on it can be tested. We did not find correlations between fundamental frequency of the first element and body weight within the adult age class but found a negative correlation in the juvenile age class. Despite this finding that indicates a tendency for juveniles to emit alarm calls of lower fundamental frequency as the animals get larger, all studied individuals increased the fundamental frequency of the first element of their alarm calls after 1 year when they became adults. Consequently, the fundamental frequency of the first element was significantly higher in adults than in juveniles despite expectations based on considerable differences in body sizes between these age classes. Vater et al. [2003] reported increasing frequency in maturing mustached bats (*Pteronotus parnellii*) and suggested that these changes were related to the reduced tension of vocal folds as a consequence of smaller force development by the controlling muscles. The expected relationship

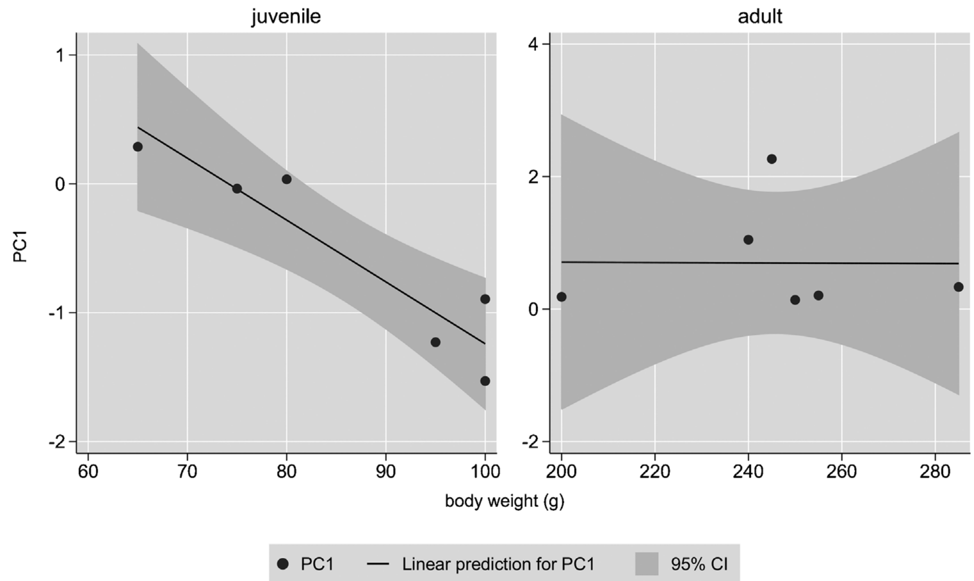

Fig. 4. Correlations between the body weight and PC1 representing the frequency parameters *start f*0, *end f*0, and *mean f*0 (factor loadings 0.87, 0.89, and 0.93, respectively) for juveniles and adults.

between body size and fundamental frequency is also absent in alarm calls of the speckled ground squirrel and yellow ground squirrel [Matrosova et al., 2007; Volodina et al., 2010]. However, in these ground squirrels, some individuals increase whereas other decrease their fundamental frequencies of the alarm calls with age, which leads to almost indistinguishable calls of juveniles and adults [Matrosova et al., 2007; Volodina et al., 2010].

The "vocal mimicry" hypothesis suggested by Matrosova et al. [2007] assumes that juvenile ground squirrels produce alarm calls structurally indistinguishable from those of adults to avoid infanticide and age-dependent predator risk. This was not unreservedly confirmed by our study. The alarm calls produced by juvenile European ground squirrels were easily distinguished, not only based on significant differences in acoustic parameters but primarily by the absence of the second element. As discussed above, juveniles may not be able to produce the second element. However, we cannot exclude the possibility that they are fully capable of emitting complete alarm calls from their first emergence but avoid vocalizing the second frequency modulated element that can be easily localized by predators or conspecifics with infanticide tendencies [Leger and Owings, 1978; Sloan et al., 2005; Halfwerk et al., 2014].

The acoustic structure of juvenile and adult alarm calls recorded within one recording session manifested significant short-term individual differences, but multivariate statistical procedures showed that the acoustic structure of the first element was considerably different after 1 year. In one female (ID 418047), cross-validation reached high correct classification success most likely because the frequency parameters of her juvenile alarm calls were the least different from those of adults. The vast majority of alarm calls of other adult ground squirrels were misclassified as originating from this female. Nonetheless, it seems that the second female (ID 418050) with high cross-validated classification success really underwent low changes in her alarm calls during the year.

TABLE 4. Percentages of correct classification of first elements of alarm calls to six European ground squirrels with discriminant function analyses (DFA) as juveniles and after 1 year as adults and cross-validation results

	Juvenile (First-year DFA)		Adult (Second-year DFA)		Cross-validation	
Individual	*n*	%	*n*	%	*n*	%
Male 420670	15	86.7	11	27.3	11	0.0
Male 418114	15	80.0	15	100.0	15	6.7
Male 418091	15	93.3	15	46.7	15	0.0
Female 420663	15	93.3	12	58.3	12	0.0
Female 418047	14	100.0	11	100.0	11	100.0
Female 418050	15	80.0	10	100.0	10	60.0
Total	89	88.8	74	71.6	74	24.3

n, number of alarm calls.

The present study clearly shows that the acoustic structure of the alarm calls of six juvenile European ground squirrels from a semi-captive enclosure was significantly changed after 1 year and that the calls underwent nearly the same changes in all individuals. However, this study is based on one re-capture after 1 year per individual and cannot determine exactly whether these changes occurred prior to or after the hibernation. Moreover, although the frequency parameters were higher in larger adults than in smaller juveniles, we found that larger juveniles emitted alarm calls with lower frequency parameters than smaller juveniles. That is also highly clamoring for further investigations on early ontogeny of alarm calls in the European ground squirrel. Another interesting question is whether the pronounced age-dependent differences are equally present in free-ranging populations. Captive or semi-captive populations may exhibit different behaviors from those of wild animals because of different environmental conditions [Mateo and Holmes, 1999]. Ground squirrels living in a completely enclosed enclosure at the Prague Zoo definitely experience lower predator pressure, but the extremely high density of individuals in a relatively small area may lead to a higher risk of infanticide.

Unfortunately, the same study with a larger number of permanently marked individuals cannot be currently performed in the Czech Republic. Based on unpublished data obtained from the speckled and yellow ground squirrels, a sample size of 20 successfully re-captured individuals in 1 year would require the permanent marking of at least 100 individuals. The estimated number of individuals occupying the enclosure at the Prague zoo was approximately 60 individuals in 2012. The estimated total number of European ground squirrels living in free-ranging populations in the Czech Republic is approximately 3,180, with approximately 24% of localities having more than 100 individuals [Matějů et al., 2010]. Therefore, it is difficult to obtain permission to treat and permanently mark a larger number of free-ranging individuals of this critically endangered and protected species. Under these circumstances, similarly oriented research may at best proceed to studies of independent samples of different age classes [see, e.g., Matrosova et al., 2007]. We aim to proceed in this way, with a special emphasis on comparison of semi-captive and free-ranging populations.

CONCLUSIONS

1. The acoustic structure of alarm calls produced by six permanently marked juvenile European ground squirrels from a semi-natural enclosure was significantly different from the acoustic structure of alarm calls of the same individuals after 1 year, after their first hibernation, when they were adults.
2. The alarm calls of all studied ground squirrels comprised only one element with almost constant frequency as juveniles and included a second frequency-modulated element as adults.
3. Differences in the acoustic parameters measured from the first element were also found between juveniles and adults, and it is remarkable that all studied individuals underwent nearly the same changes. The most prominent differences included decreased duration and increased fundamental frequency of this element.
4. Further research may elucidate whether these structural changes in the alarm calls of the European ground squirrel occur prior to or after hibernation. Another interesting question is whether the differences between juveniles and adults are also typical for free-ranging populations.

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