Frequency-modulated second elements of two-element alarm calls do not enhance discrimination of callers in three Eurasian ground squirrels

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Abstract
Alarm calls of the European Spermophilus citellus (EGS), Taurus S. taurensis (TGS) and Anatolian S. xanthopyrýmnus (AGS) ground squirrels share the same basic structure. They are tonal sounds consisting primarily of two different elements. The first element, often produced without the second element, has limited frequency modulation, while the second element is more frequency modulated. The present study examined whether this frequency-modulated element enhances the individual distinctiveness of calls, allowing calls to be ascribed with greater confidence to individual callers of the three species. Cross-validated discriminant function analysis (DFA) based on five acoustic parameters of the first element successfully classified calls to correct individuals (EGS: 90%, TGS: 98%, AGS: 96%). Cross-validated DFA based on five acoustic parameters of the second element was also successful in classifications (EGS: 88%, TGS: 86%, AGS: 96%), though discrimination of callers based on parameters of the second versus first element was the same for the AGS, lower for the EGS and significantly lower for the TGS. Cross-validated DFA based on five acoustic parameters of two-element calls also successfully classified calls to correct individuals (EGS: 93%, TGS: 98%, AGS: 97%), though did not improve the extent to which calls could be classified to individuals beyond that based on the first element alone. Thus, the second element does not enhance the individual distinctiveness of calls, but may convey other information such as the location of the caller [Current Zoology 58 (5): 749–757, 2012].

Keywords
Alarm call, Ground squirrel, Individual recognition, Spermophilus citellus, Spermophilus taurensis, Spermophilus xanthopyrýmnus

Colonially living ground-dwelling sciurids typically emit alarm calls in response to predators (Sherman, 1977). Considerable variability has been found in the acoustic structure of these calls (e.g. Nikol'skii and Orlènev, 1980; Owings and Leger, 1980; Blumstein, 1995; Perla and Slobodchikoff, 2002; Frederiksen and Slobodchikoff, 2007; Matrosova et al., 2011). For example, in vocal repertoires of some North American ground squirrels, two structurally different types of alarm calls have been found. Tonal and high-frequency calls are primarily produced in the presence of aerial predators, while rhythmically-repeated, broadband calls are emitted in the presence of terrestrial predators (Koeppl et al., 1978; Owings and Virginia, 1978; Robinson, 1981; Davis, 1984). These different types of alarm calls most probably reflect the degree of risk and urgency of response rather than being referential (Macedonia and Evans, 1993; Blumstein, 2007). Unlike North American ground squirrels, Eurasian ground squirrels of the genus Spermophilus sensu stricto (Helgen et al., 2009) produce one type of alarm call in response to both terrestrial and aerial predators (Nikol'skii, 1979; Matrosova et al., 2012).

Three closely related Eurasian ground squirrels, the European ground squirrel Spermophilus citellus (hereinafter EGS), Taurus ground squirrel S. taurensis (hereinafter TGS) and Anatolian ground squirrel S. xanthopyrýmnus (hereinafter AGS) produce species-specific alarm calls, which share a similar basic structure (Schniederová and Policht, 2012a). These alarm calls can be repeated by individual ground squirrels in longer series, lasting as long as the alarm-evoking stimulus persists in their colony. In all three species, each single alarm call most commonly comprises two different elements, where the first element has limited frequency modulation, while the second element immediately following that is more frequency modulated (Schniederová and Policht, 2012a). Nevertheless, alarm calls containing only the second element, or more frequently, only the first element, are often produced by individuals. Such calls might be emitted as exceptions within a repeated series of calls, or as alternatives to standard
two-element calls (Schneiderová and Policht, 2010, 2012a). In the EGS, about 60% of individuals detained in live-traps produce at least some calls consisting of the first element only and about 3% of individuals exclusively produce calls consisting of the first element only (Schneiderová, unpublished data). Thus, it is evident that spectral variability exists within the one type of alarm call of the EGS, TGS and AGS. It remains unknown, however, whether the second, frequency-modulated element of alarm calls in these ground squirrels encodes significant or additional information.

In the past years, potential information encoded in alarm calls of ground-dwelling sciurids has been studied extensively (e.g. Owings and Leger, 1980; Blumstein, 1995; Sloan et al., 2005; Frederiksen and Slobodchikoff, 2007) with particular attention devoted to individuality and discrimination of callers (e.g. Hare, 1998; Blumstein and Munos, 2005; Matrosova et al., 2011; Pollard and Blumstein, 2011). Discrimination of callers may be adaptive for ground-dwelling sciurids because it enables animals in a colony to adjust their responses according to reliability of callers (reliability hypothesis) (Hare and Atkins, 2001; Blumstein et al., 2004), to assess the number of alarm calling individuals (multiple calling hypothesis) (Sloan and Hare, 2008; Matrosova et al., 2009), or to discern the direction of movement of potential predators from the emergent pattern of calling among multiple signalers (predator-tracking hypothesis) (Thompson and Hare, 2010). Under each of these hypotheses, animals reduce energetic costs and time lost from foraging and other activities by reducing responsiveness to unreliable callers (reliability hypothesis), reducing response to calls emitted by a single individual that signify less urgency of response than calls produced by multiple callers (multiple calling hypothesis), or modulating their response according to whether a predator is approaching or retreating from their present location (predator-tracking hypothesis).

Playback experiments have revealed that both yellow-bellied marmots Marmota flaviventris (Blumstein and Daniel, 2004), and Richardson's ground squirrels Urocitellus richardsonii (Hare, 1998), are able to discriminate among individual callers. A high potential for individual coding has been demonstrated in the acoustic structure of alarm calls of many ground-dwelling squirrels including the steppe marmot Marmota bobak (Nikol'skii and Suchanova, 1994), yellow-bellied marmot M. flaviventris (Blumstein and Munos, 2005), Olympic marmot M. olympus (Pollard et al., 2010), white-tailed prairie dog Cynomys leucurus (Pollard and Blumstein, 2011), black-tailed prairie dog C. ludovicianus (Pollard and Blumstein, 2011), California ground squirrel Otospermophilus beecheyi (Pollard and Blumstein, 2011), Belding's ground squirrel Urocitellus beldingi (McCowan and Hooper, 2002), Richardson's ground squirrel Urocitellus richardsonii (Pollard and Blumstein, 2011), thirteen-lined ground squirrel Ictidomys tridecemlineatus (Pollard and Blumstein, 2011), speckled ground squirrel Spermophilus suslicus (Volodin, 2005), yellow ground squirrel S. fulvus (Matrosova et al., 2010), and also in the EGS and TGS (Schneiderová and Policht, 2010). Individual distinctiveness of alarm calls might be also expected in the AGS, as its alarm calls share the same basic structure of those of the EGS and TGS (Schneiderová and Policht, 2012a).

Schneiderová and Policht (2010) reported extensive spectral variability in the second frequency-modulated element of alarm calls of EGS and TGS, and noted that this variability allows human observers to discriminate among individual callers based solely on a visual assessment of the spectrograms of their calls. However, high individual distinctiveness of EGS and TGS alarm calls in their study was achieved by simultaneously utilizing acoustic parameters measured from both the first and second elements of each call. Thus, it remains unclear whether there are significant differences in the potential for coding individuality in the first versus the second elements of the alarm calls of these Spermophilus species.

Frequency modulation in calls may enhance signal transmission, its localizability or expand its information content (Leger and Owings, 1978; Owings and Hennessy, 1984; Bradbury and Vehrencamp, 1998; Sloan et al., 2005). In several mammals, including bats, dolphins, wolves and pinnipeds, frequency modulation has proven to be an important parameter encoding information about caller identity (Scherrer and Wilkinson, 1997; Charrier et al., 2003; Charrier and Harcourt, 2006; Janik et al., 2006; Palacios et al., 2007; Charrier et al., 2010). In the Belding's ground squirrel, spectral structure of alarm chirps is an important factor encoding identity of callers (McCowan and Hooper, 2002). Contrary to these findings, frequency contour parameters were identified as the spectral attributes contributing the least to individual differences in alarm calls of the speckled ground squirrel, though frequency-related variables, and maximum fundamental frequency in particular, remained an important factor in assigning calls to individuals (Matrosova et al., 2009).
The primary aim of this study was to determine the relative efficacy of the first versus the second element of EGS, TGS and AGS alarm calls in encoding caller identity. Further, the present study constitutes the first test of whether the alarm calls of AGS are individually distinctive, like the structurally-similar alarm calls of EGS and TGS.

1 Materials and Methods

1.1 Study sites and animals

Two-element alarm calls of eight individual EGS were recorded from June to August in 2007 and during August 2008 in a natural colony located at the Airfield Letňany in Prague, Czech Republic (50° 07´ N, 14° 31´ E). Two-element alarm calls of eight individual TGS were recorded during August 2007 in a natural colony located at Yarpuz near Akseki, Turkey (38° 08´ N, 31° 53´ E). Two-element alarm calls of eight individual AGS were recorded during June 2009 in a natural colony located at the campus of Ercyies University in Kayseri, Turkey (38° 42´ N, 35° 32´ E).

The recorded ground squirrels were either adults, i.e. individuals who had hibernated at least once (EGS: three females, TGS: one male and one female, AGS: eight females), or juveniles, i.e. ground squirrels that had yet to undergo their first hibernation at the time of recording (EGS: four males and one female, TGS: four males and two females). For more details see Schneiderová and Policht (2012b).

1.2 Data collection

Alarm calls were recorded using a Sony MZ-RH10 digital recorder (sampling frequency 44.1 kHZ, sample bit depth 16 bit) and an Audio Technica ATR55 directional microphone (frequency response 70−18,000 Hz). Recorded alarm calls were produced toward the researcher by free-living ground squirrels that were sitting in the entrance of their burrows. The distance between the ground squirrels and the researcher with the recording equipment ranged from 5 to 10 m. Each ground squirrel was recorded during a single recording session lasting from 5 to 15 minutes. The number of calls recorded from each ground squirrel was from 30 to 230 calls. After the recording, each ground squirrel was live-trapped using a smooth brown string snare attached at the entrance of its burrow. Live-trapped ground squirrels were then weighed, their sex and approximate age were ascertained, and they were marked with black hair dye (Palette, Schwarzkopf & Henkel, 900 Black, Düsseldorf, Germany) to prevent repeated recording of the same individual. Upon completion of these procedures, ground squirrels were released at the entrance of their burrows. For further details concerning live-trapping procedures see Schneiderová and Policht (2012b).

1.3 Acoustic analysis

Alarm call recordings were analyzed using Avisoft SASLab Pro software, version 5.1. From each individual of the 8 individuals of the three species, 30 alarm calls containing both elements of the highest quality, i.e. with a good signal to noise ratio, were selected for detailed analysis. In total, 720 alarm calls were analyzed (240 per species). Alarm calls were manually separated and labeled with the help of the waveforms and spectrograms of following parameters: Hamming window, FFT length 512, frame size 50% and overlap 93.75%. These settings provided a frequency resolution of 86 Hz, a time resolution of 0.73 s and a bandwidth of 224 Hz. All acoustic parameters were measured from the fundamental frequency band (f0) using the “Automatic parameter measurements” tool. From each of the two elements of each alarm call, five acoustic parameters were measured (Fig. 1), including the duration (dur), starting frequency (start), ending frequency (end), the frequency of maximum amplitude (peak) and the relative standard deviation of the elements (std). To measure the relative standard deviation, the frequency of maximum amplitude was computed for all spectra between the start and the end of each element. Then, the relative standard deviation was calculated by the software as a quotient between the standard deviation and the mean value. This parameter was used for quantifying frequency modulation (Specht, 2010). Parameters dur, start, end and peak also strongly reflect individual differences in the basic contour of elements, especially of the second element (Fig. 2).

Fig. 1Measured acoustic parameters marked on a representative spectrogram of the alarm call of the European ground squirrel Spermophilus citellus

Standard deviations of the first (std1) and the second (std2) elements, are not shown.
Fig. 2  Representative spectrograms of alarm calls of eight different individuals of (A) the European ground squirrel *Spermophilus citellus*, (B) the Taurus ground squirrel *S. taurensis* and (C) the Anatolian ground squirrel *S. xanthopymnus*

1.4 Statistic analysis

Standard discriminant function analysis (DFA) was applied to all three species studied to estimate the percentage of classification to correct individuals. For each individual caller, the data set consisting of ten measured acoustic parameters (five from the first and five from the second element) measured from 30 calls was separated into two separate data sets containing 15 calls. Data from the first 15-call dataset were used to perform two separate DFAs, the first to estimate the percentage of correct classification for the first element and the second to estimate the percentage of correct classification for the second element. Data from the second 15-call dataset were used to perform DFA to estimate the percentage of correct classification for two-element calls. Because correct classification in this DFA was likely to be enhanced by doubling the number of acoustic parameters used (five from the first element and five from the second element), the number of acoustic parameters that entered this analysis was reduced to include only the five parameters that best contributed to the discrimination, i.e. five parameters with the largest F-values, were selected to perform DFA based on both elements. In all three species, values were normally distributed for approximately half of acoustic parameters (Kolmogorov-Smirnov test), though DFA is relatively robust to violations of this assumption, and thus the method was applied to all variables for each species. To validate results of all DFAs, a leave-one-out cross-validation procedure was performed (Huberty, 1994). All DFAs and cross-validation procedures were performed in SPSS, version 13.

To compare values of correct classifications resulting from cross-validated DFAs for calls containing the first element only and calls containing the second element only, Yates corrected $2 \times 2$ chi-square tests were used. The same test was used to compare values of correct classifications resulting from cross-validated DFAs for calls containing the first element only and two-element calls. These tests were performed in STATISTICA, version 6.0.

In all three species, the potential for individual coding (PIC) for each measured acoustic parameter was also calculated as the ratio CVb/meanCVw, where CVw was the within-individual coefficient of variation and CVb was the between-individual coefficient of variation. Coefficients of variation were calculated using the formula $CV = 100 \times (SD/\bar{x})$, where SD was the standard deviation and $\bar{x}$ was the mean of the sample. PIC value > 1 indicate that the intra-individual variability is less than the inter-individual variability, and thus that the parameter may be used for individual recognition. PIC values > 2 suggest that the parameter is highly individualized (Robisson et al., 1993; Charrier et al., 2010).

2 Results

Three DFAs and cross-validated DFAs were performed for each species of ground squirrel. The first DFA was based on five acoustic parameters measured from the first element only, the second was based on five acoustic parameters measured from the second element only and the last one was based on five acoustic parameters measured from both elements. Results of these DFAs and cross-validated DFAs are summarized in Table 1. In general, classification success was high (> 80%) for all DFAs and cross-validated DFAs in all the three species (Table 1, Fig. 3).
Table 1  Results of discriminant function analyses (DFA) performed separately for alarm calls consisting of the first element only, the second element only, or both the first and second elements

<table>
<thead>
<tr>
<th>Acoustic parameters entering the analysis</th>
<th>EGS</th>
<th>TGS</th>
<th>AGS</th>
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<tbody>
<tr>
<td></td>
<td>1st element</td>
<td>2nd element</td>
<td>Both elements</td>
</tr>
<tr>
<td>dur1</td>
<td>dur2</td>
<td>dur1</td>
<td></td>
</tr>
<tr>
<td>start1</td>
<td>start2</td>
<td>end1</td>
<td></td>
</tr>
<tr>
<td>end1</td>
<td>end2</td>
<td>dur2</td>
<td></td>
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<tr>
<td>peak1</td>
<td>peak2</td>
<td>start2</td>
<td></td>
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<tr>
<td>std1</td>
<td>std2</td>
<td>end2</td>
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</table>

| DFA | 93.3% | 94.2% | 97.5% | 100% | 87.5% | 100% | 96.7% | 96.7% | 97.5% |
| Cross-validated DFA | 90.0% | 88.3% | 92.5% | 98.3% | 85.8% | 98.3% | 95.8% | 95.8% | 96.7% |
| Wilks’ lambda | 0.000605 | 0.002490 | 0.000148 | 0.000052 | 0.000287 | 0.000003 | 0.000091 | 0.000428 | 0.000092 |

Function 1

| Variance explained by Function 1 | 82.1% | 74.4% | 55.6% | 73.1% | 55.5% | 62.5% | 85.9% | 49.9% | 63.3% |
| Correlation with Function 1 | end1 | start2 | end1 | start1 | end2 | start1 | end1 | start2 | std1 |
| Correlation coefficient (r) | 0.87 | 0.88 | 0.90 | 0.87 | 0.78 | 0.89 | 0.43 | -0.80 | 0.63 |

Function 2

| Variance explained by Function 2 | 12.9% | 11.8% | 23.5% | 19.4% | 22.3% | 29.8% | 12.5% | 39.4% | 22.9% |
| Correlation with Function 2 | dur1 | end2 | dur1 | dur1 | peak2 | dur2 | dur1 | end2 | dur1 |
| Correlation coefficient (r) | 0.89 | 0.55 | 0.54 | 0.70 | 0.93 | 0.55 | 0.76 | 0.80 | 0.82 |

Abbreviations: EGS, European ground squirrel *Spermophilus citellus*; TGS, Taurus ground squirrel *S. taurensis*; AGS, Anatolian ground squirrel *S. Xanthoprymnus*.

In the EGS, the classification success of the cross-validated DFA did not differ significantly between calls containing the first element only and calls containing the second element only ($\chi^2 = 0.04, df = 1, P = 0.84$). Classification success of the cross-validated DFA for two-element calls was slightly but not significantly higher than that for calls containing the first element only ($\chi^2 = 0.21, df = 1, P = 0.65$). In the TGS, classification success of the cross-validated DFA was significantly lower for calls containing the second element only than for calls containing the first element only ($\chi^2 = 11.20, df = 1, P = 0.0008$). Classification success of the cross-validated DFA for two-element calls did not differ from that based on the first element only ($\chi^2 = 0.25, df = 1, P = 0.61$). In the AGS, classification success of the cross-validated DFA was the same for calls containing the first element only and those containing the second element only ($\chi^2 = 0.10, df = 1, P = 0.75$). Classification success of the cross-validated DFA for two-element calls was not significantly higher than for calls containing the first element only ($\chi^2 = 0.00, df = 1, P = 1.00$).

All acoustic parameters measured from alarm calls of the EGS, TGS and AGS had PIC values > 1. The highest potential for individual coding (PIC values > 4) was evident among frequency parameters of the first element (*start1, end1, peak1*) (Table 2).

### 3 Discussion

This study showed that both the first and second elements of EGS, TGS and AGS alarm calls have the potential to encode information about individuality. However, the discrimination of individual callers based on acoustic parameters of the second element was the same for the AGS, lower for the EGS and significantly lower for the TGS than discrimination based on parameters derived from the first element. The reduced discrimination efficiency of the second element could be explained by higher within-individual variability of this element relative to the first element (Table 2). The most crucial finding of this study was that only the first element, often emitted in natural calls without the second element, was sufficient to allow discrimination among callers and that the inclusion of the second element did not improve the extent to which calls could be classified to individuals beyond that based on the first element alone. In all three species, the most individualistic acoustic parameters were the frequency parameters of the first element (Table 2). In addition, DFA based on
Fig. 3  Scatterplots showing separation of eight individuals of (A) the European ground squirrel *Spermophilus citellus*, (B) the Taurus ground squirrel *S. taurensis* and (C) the Anatolian ground squirrel *S. xanthopyrnu*ns.

Individually were separated by the first two discriminant functions generated by discriminant function analysis based on five acoustic parameters measured on the first element only (left) and based on acoustic parameters measured on both elements of the alarm call (right).

Acoustic parameters of the first element classified more than 90% of calls to correct individuals in all the three species. Volodin (2005) also achieved high discrimination success (96%) among the relatively simple alarm calls of the speckled ground squirrel. Speckled ground squirrel alarm calls do not contain any frequency modu-
Table 2  Coefficients of variation and values of potential for individual coding (PIC) of measured acoustic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGS</th>
<th></th>
<th></th>
<th>TGS</th>
<th></th>
<th></th>
<th></th>
<th>AGS</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>CVb</td>
<td>CVw</td>
<td>PIC</td>
<td>CVb</td>
<td>CVw</td>
<td>PIC</td>
<td>CVb</td>
<td>CVw</td>
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<tr>
<td>dur1</td>
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<td>2.11</td>
<td>19.53</td>
<td>8.69</td>
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</table>

Abbreviations: EGS, European ground squirrel *Spermophilus citellus*; TGS, Taurus ground squirrel *S. taurensis*; AGS, Anatolian ground squirrel *S. xanthopyrnus*; CVb, between-individual coefficient of variation; CVw, mean within-individual coefficient of variation; PIC, potential for individual coding.

As previously demonstrated for alarm calls of the EGS and TGS (Schneiderová and Policht, 2010), the present study revealed that the alarm calls of AGS encode information regarding caller identity. In general, the alarm calls of ground-dwelling sciurids manifest

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Related element, unlike the alarm calls of the EGS, TGS and AGS as well as the alarm calls of the pygmy ground squirrel *Spermophilus pygmaeus*, and Alashan ground squirrel *S. alashanicus* (Nikol'skii, 1979). Thus, even simply-structured alarm calls with almost constant frequency are sufficient to allow discrimination among individual signalers. In spite of that, the possible adaptive significance of high individual distinctiveness of the second element is not excluded by this study. In the EGS, a small number of individuals in the live-traps (about 3%) produce calls containing the second element only instead of the standard two-element calls (Schneiderová, unpublished data). Such frequency-modulated elements in the alarm calls of Eurasian ground squirrels may convey redundant information regarding individual identity, or information beyond that conveyed by the first element.

Frequency modulated elements can be also found in alarm calls of some North American ground squirrels, which have been studied in more detail. In Richardson's ground squirrels, frequency modulated elements sometimes follow the primary syllable of repetitive alarm calls, produced primarily in the presence of terrestrial predators (Koeppel et al., 1978; Sloan et al., 2005). Sloan et al. (2005) found that inclusion of this element increases with proximity of the caller to the stimulus eliciting the alarm call. Moreover, playback experiments revealed that the addition of this element promotes increased and lasting vigilance of alarm call recipients and facilitates the orientation of the receiver to the signaler (Sloan et al., 2005). In the California ground squirrel *Otospermophilus beecheyi*, vocal responses to predators constitute a graded system of structurally variable alarm calls, which, among other parameters, vary in terms of the presence of frequency modulation (Leger and Owings, 1978; Owings and Virginia, 1978). Frequency modulated elements are included in single-note or repetitive alarm calls produced by highly excited California ground squirrels, mostly toward terrestrial predators (Leger and Owings, 1978; Owings and Virginia, 1978). Playback experiments showed that calls containing frequency modulation are more easily localized by humans than calls without frequency modulation (Owings and Virginia, 1978). California ground squirrels also react differently to repetitive calls containing frequency modulation versus single-note calls without frequency modulation. These differences are probably related to the fact that repeated calls in this species are elicited primarily by terrestrial predators, while single-note calls are elicited by raptors (Leger and Owings, 1978). The context surrounding alarm call production, i.e., type of predator encountered, risk of situation or distance from the alarm-evoking stimulus should be studied in more detail in EGS, TGS or AGS to reveal whether such aspects influence production of structurally different alarm calls. Moreover, playback experiments should be conducted to test whether these ground squirrels react differently to structurally different alarm calls. In contrast to some North American ground squirrels, these aspects of alarm calling behavior have never been studied in these Eurasian ground squirrels.

As previously demonstrated for alarm calls of the EGS and TGS (Schneiderová and Policht, 2010), the present study revealed that the alarm calls of AGS encode information regarding caller identity. In general, the alarm calls of ground-dwelling sciurids manifest
high levels of individual distinctiveness (Nikol’skii and Suchanova, 1994; McCowan and Hooper, 2002; Blumstein and Munos, 2005; Volodin, 2005; Matrosova et al., 2010; Schneiderová and Policht, 2010; Pollard and Blumstein, 2011). However, alarm calls in the present study and some previous studies were probably recorded only once during a single recording session from all the individuals (McCowan and Hooper, 2002; Volodin, 2005; Schneiderová and Policht, 2010), thus confounding context and signaler identity. In other words, it is possible that situation-specific information, also encoded in alarm calls of some ground-dwelling sciurids (Owings and Leger, 1980; Frederiksen and Slobodchikoff, 2007; Slobodchikoff et al., 2009), may have been incorrectly interpreted as information about caller individuality. Moreover, recent long-term studies showed that individuality of alarm calls in speckled and yellow ground squirrels is unstable over time (Matrosova et al., 2009, 2010). Therefore, long-term studies on the acoustic structure of alarm calls in the EGS, TGS and AGS should be conducted to reveal whether there is additional situation-specific information encoded in their calls, or whether the acoustic structure of their calls changes over time. Such research would not only provide a better understanding of the adaptive significance of individually-distinct alarm calls among ground squirrels, but would also explore the potential utility of these alarm calls for noninvasive identification of individuals (McCowan and Hooper, 2002; Pollard et al., 2010; Schneiderová and Policht, 2010).

Beyond these gaps in our knowledge, there is no information about the possible sex- and age-related variability in alarm calls of the EGS, TGS and AGS. Mixed samples of individuals, including males and females as well as juveniles and adults, were used in the present and previous studies (Schneiderová and Policht, 2010). Therefore, some of the distinctiveness of alarm calls in these species might also be attributed to sex- and age-related differences among callers. However, AGS calls in the present study were all obtained from adult females, and those were not less individually-distinct than calls of either EGS or TGS where calls of both adult and juvenile males and females were recorded. Matrosova et al. (2011) showed that in alarm calls of speckled ground squirrels, yellow ground squirrels and yellow-bellied marmots, structural features encoding individuality are more pronounced than those encoding sex or age. Further, for both speckled and yellow ground squirrels, calls of juveniles were not distinguishable from calls of adults (Matrosova et al., 2007), just as Swan and Hare (2008) demonstrated that signaler age does not affect receiver responsiveness to individually-distinguishable Richardson's ground squirrel alarm calls. Taken together, such findings indicate that acoustic cues to individuality are generally encoded more strongly than those related to signaler sex or age in alarm calls of ground-dwelling sciurids, emphasizing the adaptive value of such discrimination in the context of the reliability, multiple calling and the predator-tracking hypotheses. Both the relative strength of individual-, age-, and sex-specific information encoded in alarm calls, and the extent to which individual discrimination functions to promote reliability detection, enumeration of callers or to facilitate predator tracking remain untested in the three Eurasian ground squirrel species examined in the present study.

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