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# SHORT-TERM AND LONG-TERM INDIVIDUALITY IN SPECKLED GROUND SQUIRREL ALARM CALLS

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Apart from the alerting function of alarm calls, selection may favor cues that help individuals to distinguish between reliable and unreliable callers. However, this mechanism for selective response to real and false alarms may act only if the individual characteristics of the call are stable at least for some time. Here we test this implicit assumption for the caller's reliability hypothesis, studying individuality of alarm calls in a colony of free-living, individually marked speckled ground squirrels (*Spermophilus suslicus*). We recorded each of 20 study animals 4 times during repeated captures when calling from a live trap toward a human, with spans of 1 day, 2 weeks, and 1 year from the 1st capture. Ten alarm call notes per animal per capture were analyzed. Individual alarm call notes showed high similarity within captures but differed strongly between captures. Both multivariate analysis of variance and discriminant function analysis showed that vocal individuality decreased rapidly with an increase of the time span between recordings. However, vocal individuality always remained higher than expected random value. Examination of our data suggests that alarm calls are unstable, which contradicts the caller reliability hypothesis, because its implicit assumption of stable individual identity is not fulfilled. However, short-term stability still may be sufficient to ensure short-term individual recognition between kin and neighbors. Also, even if the alarm calls change structurally, because group members meet up daily, they can update their knowledge of the call structure of individuals, and this would likely allow them to distinguish between reliable and unreliable individuals.

Key words: alarm call, caller reliability, speckled ground squirrel, *Spermophilus suslicus*, temporal stability, vocal communication

One particular question of identity-focused research in bioacoustics is whether cues to individual identity are encoded in alarm calls. Unlike individual-specific long-range calls, whose function is to attract mates and to deter rivals (e.g., Conner 1985; Frommolt et al. 2003; Reby et al. 1998; Zimmermann and Lerch 1993) or to ensure parent–offspring recognition (e.g., Charrier et al. 2002; Insley et al. 2003; Torriani et al. 2006), the function of individuality in alarm calls is less obvious (Blumstein et al. 2004). Encoding individual identity in alarm contexts may be adaptive if receiver vigilance and response urgency depend on the dominance status, reliability, or kinship of the alarm signaler (Yorzinski et al. 2006).

Ground-dwelling sciurids warn kin of potential predators through alarm calls (Macedonia and Evans 1993; Owings and Virginia 1978; Sherman 1977, 1981) and represent a traditional model for testing the role of individuality in alarm calls. Individual identity encoded in the structure of alarm calls has been reported for steppe marmots (*Marmota bobak*—Nikol'skii and Suchanova 1994), yellow-bellied marmots (*M. flaviventris*—Blumstein and Munos 2005), Belding's ground squirrels (*Spermophilus beldingi*—McCowan and Hooper 2002), and speckled ground squirrels (*S. suslicus*—Volodin 2005).

The main relevant hypothesis here is the reliability of a caller for the listener (Blumstein et al. 2004; Cheney and Seyfarth 1988; Hare and Atkins 2001). Presumably unreliable young individuals or adults with low excitation thresholds are more likely to produce false alarm calls in response to harmless animals (Hare and Atkins 2001; Nesterova 1996; Robinson 1981) or in response to age-dependent risks (Hanson and Coss 1997). Because listeners should interrupt their current activity in response to alarms, too many false alarms could be costly. Devaluing information encoded in alarm calls emitted by relatively unreliable callers would benefit the listener, reducing disruptions in time spent foraging, grooming, burrowing, or in social interactions (Blumstein 2007; Fitch and Hauser 2002; Sloan and Hare 2006).

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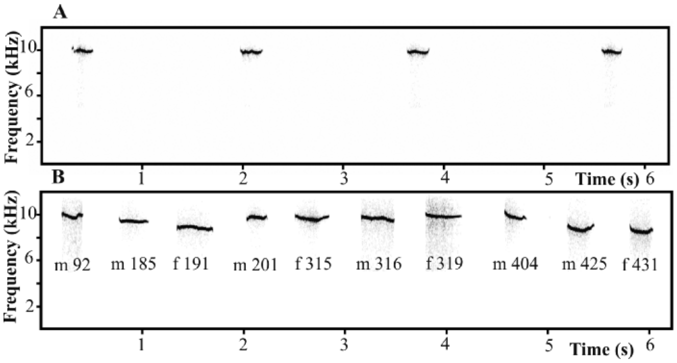

FIG. 1.—A) Spectrogram of a spontaneous series of the alarm call notes produced by male speckled ground squirrel (*Spermophilus suslicus*) no. 446 calling from a live trap toward a human. B) Spectrogram of 10 alarm call notes recorded from 10 study animals, 1 note per animal. m = males, f = females; numbers denote individual marks.

Discrimination between familiar and unfamiliar vocalizations has been observed in Richardson's ground squirrels (*Spermophilus richardsonii*—Hare 1998) and yellow-bellied marmots (Blumstein and Daniel 2004). Discrimination between calls of juveniles and adults has been observed in California ground squirrels (*S. beecheyi*—Hanson and Coss 2001), steppe marmots (Nesterova 1996), and yellow-bellied marmots (Blumstein and Daniel 2004). In Richardson's ground squirrels, there is a lack of differential response of adults to calls of juveniles varying in call rate, bout length, or both (Sloan and Hare 2006), whereas in Belding's ground squirrels there is a reduced responsiveness to calls of juveniles relative to adult-produced alarm calls (Robinson 1981). If alarm calls of juveniles are less reliable than alarm calls of adults, these results were inconsistent: steppe marmots and California, Belding's, and Richardson's ground squirrels responded more to presumably ''reliable'' adult calls, whereas adult female yellow-bellied marmots responded more to presumably ''unreliable'' juvenile calls.

All the studies on discrimination between the reliable and unreliable callers are based on the implicit assumption that individual or age- or sex-related features, or both, of alarm calls are prominent and stable at least over short periods of time. High temporal variability of alarm call structure should not allow conspecifics to relate false alarms with a particular individual. However, this assumption has not yet been tested.

Speckled ground squirrels are relatively long-living (up to 6 years—Babitsky et al. 2006), small-sized (body mass 180–220 g, body length without tail 190–220 mm), diurnal, herbivorous, obligate-hibernating sciurids (Lobkov 1999). Yearlings can breed after their 1st winter (Babitsky et al. 2006). Their meadow, steppe, and crop habitats allow good visibility only at the beginning of the growing season; by mid-June grass stands are taller than a fully erect ground squirrel, seriously reducing visual perception (Lobkov 1999; Tchabovsky 2005). The population density of this species can reach up to 200 animals/ha (Lobkov 1999; Tchabovsky et al. 2005). Animals share territories, and their dominant–subordinate relations are noticeable only within the short mating period early in the spring (Lobkov 1999; Titov 2001, 2003a).

The alarm calls of the speckled ground squirrel consist of weakly modulated tonal notes about 200 ms in duration, with fundamental frequency ranging from 8,500 to 11,500 Hz (Fig. 1A), which are typically produced in series with intervals substantially longer than the duration of the notes themselves (Matrosova et al. 2006, 2007; Nikol'skii 1979; Volodin 2005). The alarm notes produced within a series are very similar to each other, but vary significantly between individuals (Volodin 2005; Fig. 1B). Previous studies suggested only slight differences between sexes in alarm call structures (Volodin 2005) and lack of age differences in the alarm call fundamental frequency as well (Matrosova et al. 2007). Therefore, in this species, recognition of a caller's reliability may be based primarily on individual features of alarm calls.

Here we test the crucial assumption of the caller's reliability hypothesis, temporal stability of individual alarms, for speckled ground squirrels. The purpose of our study was to estimate stability of individual features encoded in the structure of alarm call notes recorded from individually marked, free-living squirrels over different periods of time: over 1 day, over 2 weeks, and over 1 year.

## MATERIALS AND METHODS

*Subjects and study area.*—We recorded alarm calls from 12 male and 8 female adult (1 year old and older) speckled ground squirrels, livetrapped repeatedly on a 1-ha colony in the Moscow region, Russia (54°47′68″N, 38°42′23″E). This is one of the northernmost colonies of this species (Shekarova et al. 2003). Aboveground activity of this colony lasts 3.5–4 months annually, from early to mid-April to early to mid-August (Tchabovsky et al. 2005). The study grid is situated on a low riverbank, 50 m from the shoreline. The colony has been studied since 2001, and all animals on the study grid are individually marked with microchips (Bayer AG, Leverhusen, Germany), and repeatedly (once every 2 weeks or more often) captured in wire-mesh live traps, 30 × 10 × 10 cm, with sunflower seed bait, followed by acoustic recording and weighing (Volodin 2005). Thus, before the start of this research, all 20 study animals were familiar with capture and call-recording procedures. All methods followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

*Data collection.*—We recorded alarm calls (Fig. 1A) during repeated 3-day-long visits, every 2 weeks in May to the beginning of June 2003–2006. During each visit, we captured a subject animal 1 or more times, and for each capture call recording and other procedures were identical. Alarm calls were recorded from squirrels in wire-mesh traps within 1 h of capture. Animals emitted alarm calls toward humans spontaneously or in response to additional stimulation (movements of a handheld baseball cap). All recordings were collected by 2 human researchers (VAM or IAV), dressed similarly in camouflage suits. In live traps, the pattern of calling toward humans and structure of alarm notes were similar to those that

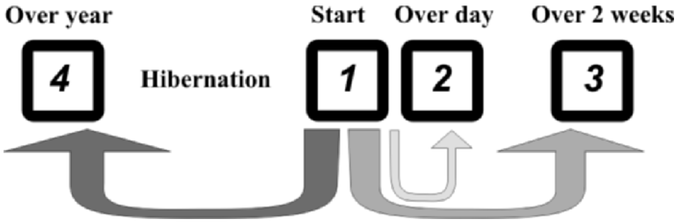

FIG. 2.—Time spans between the alarm call recordings selected for acoustic analysis for each of 20 speckled ground squirrels (*Spermophilus suslicus*). 1 = 1st recording (''start'')—the 1st recording after hibernation, made at the beginning to mid-May; 2 = 2nd recording (''over 1 day'')—the next recording after the start recording, made within 48 h from it; 3 = 3rd recording (''over 2 weeks'')—the 1st recording among recordings made 9–14 days after the start recording; 4 = 4th recording (''over 1 year'')—the 1st recording after hibernation, made a year before the start recording.

occurred under natural conditions toward predators (Matrosova et al. 2007; Nikol'skii 1979; Volodin 2005), allowing us to reliably distinguish these calls as alarms. Sound recording always preceded other manipulations of an animal (individual mark checking, physical examination, and weighing). After the manipulations, animals were released at the place of capture.

On average, a recording lasted 3–4 min and provided 30–40 alarm call notes per animal. The distance to the microphone was about 30 cm. We used a Marantz PMD-222 (D&M Professional, Kanagawa, Japan) analog tape recorder with AKG-C1000S (AKG-Acoustics Gmbh, Vienna, Austria) cardioid electret condenser microphone and Type II chrome audiocassette EMTEC-CS II (EMTEC Consumer Media, Ludwigshafen, Germany). The system provided frequency response 40–14,000 Hz on tape speed 4.75 mm/s. Additionally, we made recordings with highly sensitive equipment, a CF-recorder Marantz PMD-671 with a Sennheiser K6 ME-64 microphone (Sennheiser Electronic, Wedemark, Germany), providing a qualitative recording within a range 40–24,000 Hz, to estimate the relative amplitude of the alarm call harmonics.

All measurements of body mass (1 per animal) were made on electronic scales (Tefal Ovelys 798881; Groupe SEB, Ecully Cedex, France) with 1-g precision after an audio recording, from the same animals that provided calls for analysis.

*Call samples.*—For each of the 20 squirrels, we selected 4 call recordings with strictly specified time spans between them (Fig. 2). The 1st (''start'') recording was recorded shortly after hibernation, 2–4 weeks after emergence from burrows, that is, at the beginning to middle of May 2004, 2005, or 2006. The 2nd recording was made within 2 days ($\bar{X} = 0.8$ days ± 0.7 *SD*) of the 1st recording. The 3rd recording was made approximately 2 weeks after the start recording (from 9 to 14 days, $\bar{X} = 11 \pm 1.7$ days). The 4th recording also was made immediately after hibernation (May or the beginning of June) but it was made 1 year (2003, 2004, or 2005 depending on individual) before the start recording. We used 4th recordings made during the year preceding the start recordings to increase our sample size.

Thus, 4 recordings were made with the following time spans: from the start to the 2nd recording—less than 48 h (hereafter ''over 1 day''), from the start to the 3rd recording—9–14 days (hereafter ''over 2 weeks''), and from the start to the 4th recording—approximately 1 year (hereafter ''over 1 year'': Fig. 2). The total sample consisted of 80 recordings, 4 per animal for 20 animals. For analysis of the recordings, we randomly selected 10 alarm notes of good quality (high amplitude, not interrupted by wind) per recording (for 2 recordings we had only 3 and 7 notes), for 790 notes in total.

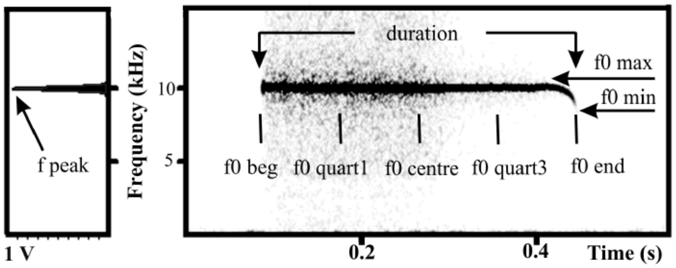

FIG. 3.—The measured temporal and frequency parameters of an alarm call note of a speckled ground squirrel (*Spermophilus suslicus*). Left: the mean amplitude spectrum; right: spectrogram. f0 max = the maximum fundamental frequency of a note, selected as maximum of 22 point measurements taken with equal intervals within a note; f0 min = the minimum fundamental frequency of a note; f0 beg = the fundamental frequency at the beginning of a note; f0 quart1 = the fundamental frequency at the 1st quarter of a note; f0 center = the fundamental frequency in the middle of a note; f0 quart3 = the fundamental frequency at the 3rd quarter of a note; f0 end = the fundamental frequency at the end of a note; f peak = the maximum amplitude frequency of a note.

*Call analysis.*—All analyses of calls were made with Avisoft SASLab Pro software version 4.3 (Avisoft Bioacoustics, Berlin, Germany). Calls were digitized with 24-kHz sampling frequency and 16-bit precision. Spectrograms were created using Hamming window, fast Fourier transform length 1,024 points, frame 50%, and overlap 96.87%. These settings provided a bandwidth of 61 Hz, frequency resolution of 23 Hz, and time resolution of 1.3 ms.

All measurements were taken from the fundamental frequency band f0, because a pilot call analysis over the frequency range up to 24 kHz showed that the fundamental frequency band coincided with frequency of maximum amplitude, that is, was always the highest in amplitude in comparison with its harmonics. Alarm call notes were weakly modulated in frequency, so it was difficult to determine a position of f0 maximum visually (Fig. 3). We used the ''automatic parameter measurements'' option of Avisoft SASLab Pro to extract the fundamental frequency values. After high-pass filtration at 1 kHz to remove background noise, we automatically measured f0 for each of 22 single power spectra, taken with equal intervals from beginning to end of a call note. Then, the f0 max, f0 min, f0 beg (point 1), f0 quart1 (point 6), f0 center (calculated as an arithmetic mean of the f0s taken in points 11 and 12), f0 quart3 (point 16), and f0 end (point 22) of a call note were automatically selected from these 22 point values. With this option, we measured duration of each alarm call note.

We also measured automatically the maximum amplitude frequency (f peak) of a call note from the mean amplitude

**Table 1.**—Multivariate analysis of variance (MANOVA) results testing the influence of individuality and interrecording span on each of 11 parameters of the alarm call notes in 20 speckled ground squirrels (*Spermophilus suslicus*); $n$ = number of notes; $F$ = $F$-ratio of MANOVA; $P$ = significance level.

| | Interrecording time span | | | | | |
|---|---|---|---|---|---|---|
| | Over day (1st and 2nd recordings) ($n = 400$) | | Over 2 weeks (1st and 3rd recordings) ($n = 393$) | | Over year (1st and 4th recordings) ($n = 397$) | |
| Call parameter | Individuality ($F$ [*d.f.* = 19, 379]) | Time ($F$ [*d.f.* = 1, 379]) | Individuality ($F$ [*d.f.* = 19, 372]) | Time ($F$ [*d.f.* = 1, 372]) | Individuality ($F$ [*d.f.* = 19, 376]) | Time ($F$ [*d.f.* = 1, 376]) |
| Duration | 45.61*** | 8.26** | 39.61*** | 1.82, $P = 0.178$ | 34.31*** | 12.69*** |
| f peak | 247.05*** | 1.57, $P = 0.211$ | 146.73*** | 1.58, $P = 0.209$ | 125.94*** | 1.35, $P = 0.246$ |
| f0 min | 189.59*** | 4.58* | 126.22*** | 0.01, $P = 0.922$ | 100.56*** | 0.38, $P = 0.540$ |
| f0 max | 348.46*** | 0.19, $P = 0.667$ | 217.10*** | 0.14, $P = 0.705$ | 128.28*** | 1.33, $P = 0.250$ |
| f0 beg | 282.03*** | 0.05, $P = 0.820$ | 179.71*** | 0.21, $P = 0.645$ | 98.31*** | 1.54, $P = 0.216$ |
| f0 quart1 | 279.14*** | 0.51, $P = 0.467$ | 166.65*** | 0.38, $P = 0.539$ | 121.59*** | 2.25, $P = 0.135$ |
| f0 centre | 258.60*** | 1.72, $P = 0.190$ | 137.44*** | 0.93, $P = 0.334$ | 125.50*** | 1.46, $P = 0.228$ |
| f0 quart3 | 259.50*** | 1.70, $P = 0.193$ | 155.21*** | 2.27, $P = 0.133$ | 130.39*** | 2.11, $P = 0.148$ |
| f0 end | 205.01*** | 4.26* | 150.18*** | 0.01, $P = 0.667$ | 119.53*** | 0.58, $P = 0.448$ |
| Up−down coefficient | 17.44*** | 4.88* | 9.37*** | 0.01, $P = 0.949$ | 11.56*** | 1.47, $P = 0.226$ |
| Extrema coefficient | 4.06*** | 4.19* | 7.39*** | 0.77, $P = 0.382$ | 4.90*** | 6.89** |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

spectrum. All measurements of duration, f0 and f peak were exported automatically to Microsoft Excel (Microsoft, Redmond, Washington).

For each alarm call note, we calculated 2 coefficients that described the fundamental frequency contour: up–down coefficient and extrema coefficient. To calculate the up–down coefficient, we estimated the direction of fundamental frequency run at each of the 22 points of measurement. An increase in frequency compared to a preceding point was marked as "+1," a decrease as "−1," and no changes as "0." The sum of these values plus 22 provided the up–down coefficient for the given note. The values of the up–down coefficient could vary from 1 (for the decreasing fundamental frequency from the 1st to the last point) to 43 (for the increasing fundamental frequency from the 1st to the last point). The extrema coefficient represented the number of local maxima and minima in the fundamental frequency contour, and could vary from 0 (with a straight line) to 20 (with a sawtooth contour).

We did not measure the interval between alarm notes because this parameter could be influenced by an observer's behavior. Overall, for each alarm call note we measured 1 temporal, 8 frequency, and 2 contour parameters.

*Statistical analyses.*—We used a 2-way multivariate analysis of variance (MANOVA) to test the influence of individuality and interrecording span on the call parameter values. We used a Friedman analysis of variance (ANOVA) to compare the individual mean parameter values for call notes of 1st, 2nd, 3rd, and 4th recordings.

We used discriminant function analysis (DFA) to calculate the probability of correctly classifying alarm call notes to the correct individual for each call sample (of 1st, 2nd, 3rd, and 4th recordings). We classified call notes from the test sets (of 2nd, 3rd, and 4th recordings) with discriminant functions derived from the training call set (of 1st recordings), considering the value of the correct cross-validation as a measure of the retained individuality with time.

We calculated the expected level of correct classification with DFA if the calls we analyzed were randomly distributed between individuals (Solow 1990; Volodina et al. 2006). For this procedure, we created 20 randomization groups from the training call set (of 1st recordings), according to the number of animals included in the DFA. Each group contained 10 calls recorded from 10 different individuals. We then applied DFA and calculated the probability of correctly classifying call notes to randomization groups. This probability was taken as a random value of classification. The differences between the random and observed values of correct classification were tested for significance with chi-square tests. All statistical analyses were made in STATISTICA, version 6.0 (StatSoft, Tulsa, Oklahoma), and differences were considered significant where $P < 0.05$.

## RESULTS

Comparison of $F$-ratios from the MANOVA indicated that the influence of individuality on call parameters was always stronger than the influence of the interrecording span (Table 1). Individuality was encoded mainly in frequency parameters, less in duration, and least in frequency contour parameters (Table 1).

Because all 3 MANOVAs included the same 20 individuals, we could compare $F$-ratios on the factor individuality for each call parameter depending on the interrecording span (Fig. 4). The comparison showed a steady decrease of individuality with an increase of the interrecording span for all frequency parameters. Individuality was pronounced at nearly equal levels in the alarm call notes recorded both over 2 weeks and over 1 year (Fig. 4). The $F$-ratio values for duration and 2 contour parameters varied slightly with increasing interrecording span. Therefore, the f0 max showed the greatest potential to encode individuality in calls, whereas frequency contour parameters showed the least (Table 1; Fig. 4).

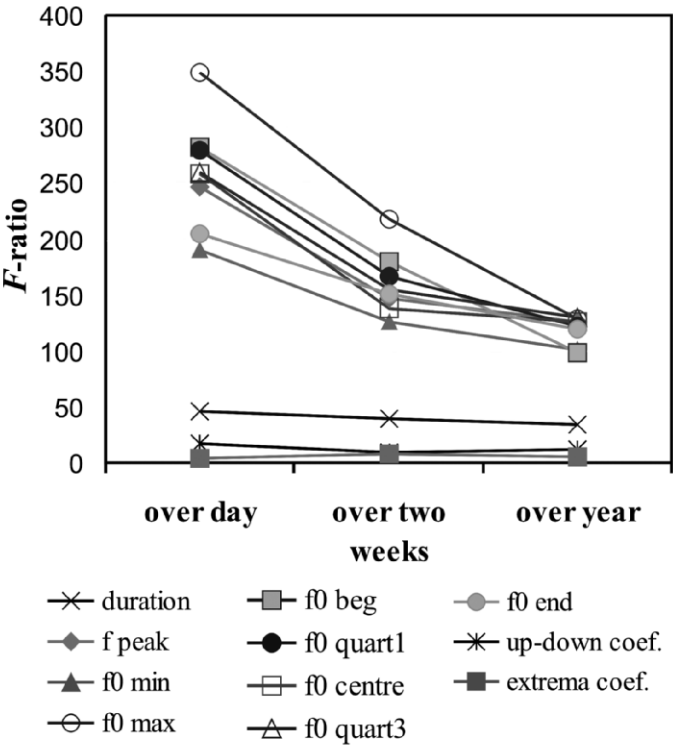

**FIG. 4.**—Multivariate analysis of variance *F*-ratio values on factor individuality for 11 parameters of the alarm call notes of 20 speckled ground squirrels (*Spermophilus suslicus*), depending on the interrecording span.

**TABLE 2.**—Friedman ANOVA results for the mean parameter values of the alarm call notes recorded from 20 speckled ground squirrels (*Spermophilus suslicus*) with different interrecording spans, and the comparative results for body masses of the same 20 speckled ground squirrels; $P$ = significance level.

| | Friedman ANOVA | |
|---|---|---|
| Call parameter | $\chi^2_3$ | *P* |
| Duration | 2.22 | 0.529 |
| f peak | 2.22 | 0.528 |
| f0 min | 4.14 | 0.247 |
| f0 max | 3.24 | 0.356 |
| f0 beg | 4.02 | 0.259 |
| f0 quart1 | 2.04 | 0.564 |
| f0 centre | 1.62 | 0.655 |
| f0 quart3 | 3.66 | 0.301 |
| f0 end | 4.56 | 0.207 |
| Up−down coefficient | 2.85 | 0.415 |
| Extrema coefficient | 4.77 | 0.189 |
| Body mass (g) | 21.98 | <0.001 |

Friedman ANOVA showed that none of the call parameters measured changed over any period of time (Table 2). This suggests an absence of trends in the call parameter values over time. To verify these results, we added body mass into the analysis (Table 2) because body mass increases constantly after emergence from hibernation (this period coincides with interrecording span "over 2 weeks"—Tchabovsky et al. 2005). As expected, Friedman ANOVA showed significant difference in body mass values (Table 2).

The DFA showed 91.5% correct assignment to individual for the training note set (1st recording), which significantly exceeded the random value (21.0%) calculated with the randomization procedure ($\chi^2 = 199.11$, *d.f.* = 1, $P < 0.001$). DFA applied separately to calls from the 2nd, 3rd, and 4th recordings showed similar high percentages of correct classification to individual: 90.0%, 90.2%, and 89.3% for the 2nd, 3rd, and 4th recordings, respectively (all differences between recordings were not significant, chi-square tests: $P > 0.05$; Fig. 5).

Cross-validation of notes from the test sets (from the 2nd, 3rd, and 4th recordings) with discriminant functions derived from the training note set (1st recording) showed a steady decline of correct assignment to individual with an increase of the interrecording span (Fig. 5). For call notes recorded over a day after the 1st recording, the correct assignment percentage falls abruptly from 91.5% to 54.0% (differences are significant, $\chi^2 = 69.06$, *d.f.* = 1, $P < 0.001$). A further increase of the interrecording span from 1 day to 2 weeks showed a further decrease of correct assignment, down to only 34.2% ($\chi^2 = 14.82$, *d.f.* = 1, $P < 0.001$; Fig. 5). However, the percentages of correct classification for call notes recorded over 2 weeks (34.2%) and over 1 year (33.0%) did not differ significantly ($\chi^2 = 0.02$, *d.f.* = 1, $P = 0.89$; Fig. 5). Nevertheless, for all cross-validation procedures, the percentage of correctly classified calls significantly exceeded the random value (over 1 day: $\chi^2 = 45.07$, *d.f.* = 1, $P < 0.001$; over 2 weeks: $\chi^2 = 7.93$, *d.f.* = 1, $P < 0.01$; over 1 year: $\chi^2 = 6.66$, *d.f.* = 1, $P < 0.01$).

Therefore, individuality of the alarm call notes falls abruptly already over 1 day and continues to fall through the 2 subsequent weeks. However, the low values achieved over 2 weeks were already comparable with those presented over 1 year, and a further decline did not occur. Nevertheless, some degree of

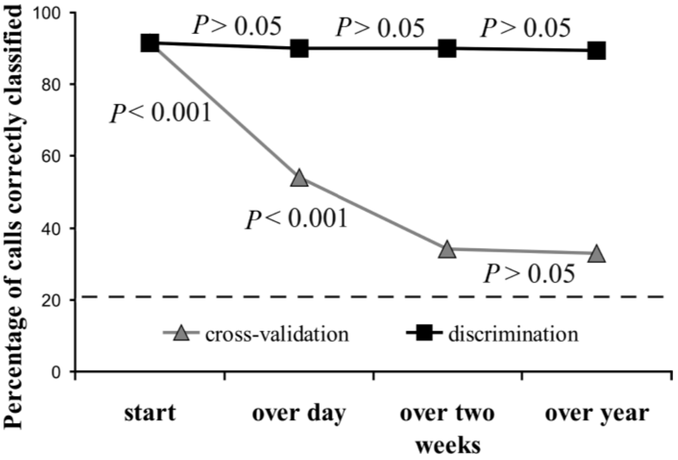

**FIG. 5.**—Mean percentages of the alarm call notes correctly classified to individual with discriminant (gray triangles) and cross-validation (dark squares) analyses for 20 speckled ground squirrels (*Spermophilus suslicus*) examined. Significance level ($P$) shows results for comparison between correct assignment values for adjacent records (with chi-square test). Dashed line shows the random value of correct classification to individual, calculated with randomization procedure (see text for description).

**Table 3.**—Percentages of alarm call notes correctly classified to individual with discriminant function analysis (DFA) and cross-validation analyses for 20 speckled ground squirrels (*Spermophilus suslicus*); $n$ = number of notes.

| | DFA | | Cross-validation analysis | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st recording (start) | | 2nd recording (over 1 day) | | 3rd recording (over 2 weeks) | | 4th recording (over 1 year) | |
| Individual | *n* | % | *n* | % | *n* | % | *n* | % |
| Male 92 | 10 | 100.0 | 10 | 0.0 | 10 | 0.0 | 10 | 0.0 |
| Female 100 | 10 | 100.0 | 10 | 0.0 | 10 | 0.0 | 10 | 20.0 |
| Male 147 | 10 | 90.0 | 10 | 100.0 | 10 | 90.0 | 10 | 90.0 |
| Male 153 | 10 | 100.0 | 10 | 60.0 | 10 | 0.0 | 10 | 0.0 |
| Male 185 | 10 | 60.0 | 10 | 80.0 | 10 | 10.0 | 10 | 20.0 |
| Female 191 | 10 | 100.0 | 10 | 40.0 | 10 | 100.0 | 10 | 100.0 |
| Male 194 | 10 | 80.0 | 10 | 70.0 | 10 | 0.0 | 10 | 40.0 |
| Male 201 | 10 | 80.0 | 10 | 10.0 | 10 | 40.0 | 10 | 0.0 |
| Male 274 | 10 | 100.0 | 10 | 90.0 | 10 | 0.0 | 10 | 80.0 |
| Male 284 | 10 | 100.0 | 10 | 60.0 | 3 | 0.0 | 10 | 10.0 |
| Female 303 | 10 | 90.0 | 10 | 60.0 | 10 | 60.0 | 7 | 14.3 |
| Female 315 | 10 | 100.0 | 10 | 70.0 | 10 | 90.0 | 10 | 0.0 |
| Male 316 | 10 | 70.0 | 10 | 80.0 | 10 | 50.0 | 10 | 80.0 |
| Female 319 | 10 | 90.0 | 10 | 30.0 | 10 | 0.0 | 10 | 30.0 |
| Female 395 | 10 | 100.0 | 10 | 40.0 | 10 | 10.0 | 10 | 0.0 |
| Male 404 | 10 | 90.0 | 10 | 80.0 | 10 | 100.0 | 10 | 0.0 |
| Male 425 | 10 | 100.0 | 10 | 50.0 | 10 | 80.0 | 10 | 100.0 |
| Male 427 | 10 | 100.0 | 10 | 30.0 | 10 | 0.0 | 10 | 0.0 |
| Female 431 | 10 | 100.0 | 10 | 80.0 | 10 | 0.0 | 10 | 20.0 |
| Female 432 | 10 | 80.0 | 10 | 50.0 | 10 | 30.0 | 10 | 50.0 |
| Total | 200 | 91.5 | 200 | 54.0 | 193 | 34.2 | 197 | 33.0 |

individuality was retained in call notes, because even over 1 year percentages of correctly classified calls exceeded the random value.

The correct classification percentages for calls from the training set (1st recording) were very high and varied from 60% to 100% between individuals (Table 3). However, cross-validation of call notes recorded even with the minimum interrecording span (over 1 day from the training set) showed that correct classification values exceeded the random value in only 17 of 20 animals (Table 3). The number of individuals showing percentages of correctly classified calls exceeding the random value decreased gradually with an increase of the interrecording span (Table 3) and on the whole corresponded to the gradual decrease of the mean percentage of correct classification (Fig. 5). Only for 3 males (m147, m316, and m425) could calls be distinguished from the total call sample with a high probability ($\geq$50%), despite the interrecording span value. For all other individuals, call structures changed substantially with time, and they were not distinguishable from the total call sample (Table 3; Fig. 6).

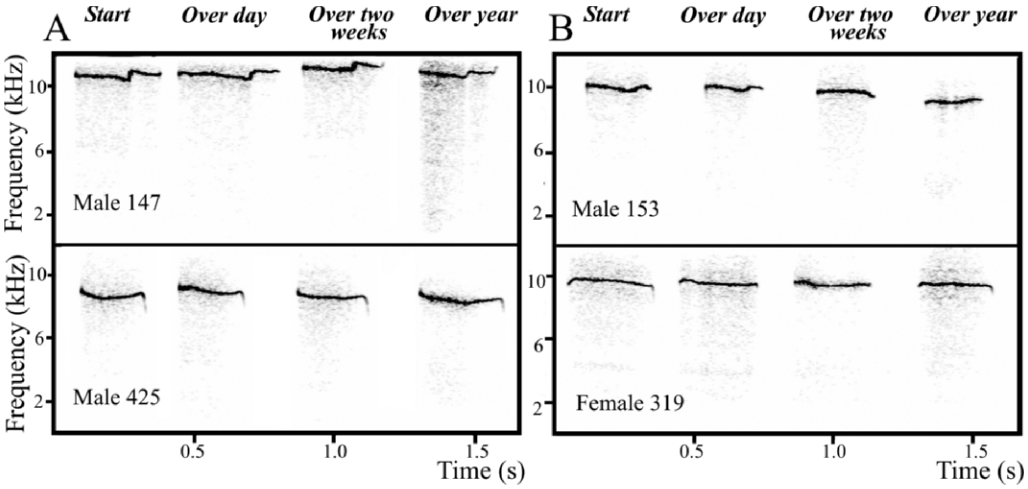

**Fig. 6.**—Spectrogram of the alarm call notes recorded from 4 speckled ground squirrels (*Spermophilus suslicus*) during 4 different captures with live traps, separated with time spans of 1 day, 2 weeks, and 1 year. A) Two individuals showing stable call structures. B) Two individuals showing unstable call structures.

## DISCUSSION

Our study showed that the alarm call notes of individual speckled ground squirrels were very similar within recordings, providing high momentary (within 1 alarm call series) individuality. Between recordings, calls of the same individual differed noticeably, showing a strong decrease in individuality with an increase in time span. This decrease in individuality was so abrupt that the levels of correct assignment to individual over 2 weeks did not differ significantly from those compared over 1 year. Nevertheless, even the lowest values were higher than a random value.

Examination of our data only partly supports the caller's reliability hypothesis for the speckled ground squirrel, because its implicit assumption of stable individual identity was not fulfilled. Given that there is little difference between sexes in the structure of alarm call notes (Volodin 2005) and lack of age differences in fundamental frequency of the notes (Matrosova et al. 2007), speckled ground squirrels are unlikely to be able to estimate a caller's reliability on the basis of long-term individual vocal identity (see also Blumstein 2007). However, speckled ground squirrels did show high short-term individuality in alarm calls. Because individuals in groups are likely to interact on a daily basis, they may be able to update changes in the call structure of different group members. Thus, short-term stability may be sufficient to ensure individual recognition between kin and neighbors.

High momentary (within a series) individuality in the calls of speckled ground squirrels could provide information on the urgency of response to a predator's presence. Calls from multiple callers should evoke a more urgent response than calls from a single caller. Such a mechanism appears to function in yellow-bellied marmots and Richardson's ground squirrels (Blumstein et al. 2004; Sloan and Hare 2006), and has been confirmed via computer modeling (Beauchamp and Ruxton 2007). Most likely, the high momentary individuality of alarm calls allows speckled ground squirrels to estimate the number of callers producing alarms simultaneously.

For speckled ground squirrels, there are only 2 short periods of the aboveground activity period when supporting individuality in calls and call-based personalized relations may be adaptive. The 1st is the breeding period, lasting about 2 weeks just after females emerge from hibernation, when males compete for mates and establish dominant–subordinate social relations (Lobkov 1999; Titov 2003a). Individual identification of a caller by its alarm call may help males to escape aggressive encounters and lower the risk of injury. The 2nd period (approximately 1 month long) is between juvenile emergence from the natal burrow and juvenile dispersal (Lobkov 1999; Titov 2003b). Selective response to calls of juveniles and enhanced calling rate in females whose litters recently emerged to the surface can represent some kind of parental care, supported by kin selection, as has been reported for yellow-bellied marmots (Blumstein and Daniel 2004; Blumstein et al. 1997).

Speckled ground squirrels are small-sized sciurids, living in tall, dense grasses (Lobkov 1999; Tchabovsky 2005) that limit their visibility within a month after they emerge from hibernation. Poor visibility prevents the listener from distinguishing callers visually and thus evaluating their potential reliability from visual identification. Such limited visibility could decrease the range of predator detection by squirrels, which should increase the response of individuals to any alarm calls because the costs of ignoring the threat are very high. However, responding urgently to any anonymous caller does not mean that alarm calling is perfectly altruistic; evidence from other *Spermophilus* supports the hypothesis that callers warn closely related philopatric kin (Sherman 1981). Therefore, despite the potential for speckled ground squirrel alarm calls to encode individual identity, selection pressures may not support the long-term sustainability of well-expressed individuality in the alarm calls.

At the same time, some degree of individuality above the random value was still present even after 1 year. These data are consistent with the finding that European ground squirrels (*Spermophilus citellus*) discriminate after hibernation between odors of familiar and nonfamiliar conspecifics (Millesi et al. 2001) and overwintered Belding's ground squirrels discriminate between odors of littermates and familiar nonkin (Mateo and Johnston 2000). Retention of vocal individuality also may promote the restoration of individual social relations after hibernation.

Unstable vocal identity also has been reported for other mammals and birds. The "boom" calls of wild male great bitterns (*Botaurus stellaris*) were stable within a day, but not over 2 weeks (Puglisi and Adamo 2004). Wild male common loons (*Gavia immer*) that switched their territories between years also changed their individually distinctive "yodel" calls (Walcott et al. 2006). Both wild and captive bald eagles (*Haliaeetus leucocephalus*) lacked stable features in chatter calls either within or between years (Eakle et al. 1989). The 2-syllable calls of captive male red-breasted geese (*Branta ruficollis*) and the barks of domestic dogs (*Canis familiaris*) varied among individuals in between-year stability (Chulkina et al. 2006; Volodin et al. 2007). The "phee" calls of a few captive marmoset species were unstable due to changes in social surrounding (Jones et al. 1993; Rukstalis et al. 2003; Snowdon and Elowson 1999) and over time (Jorgensen and French 1998). In contrast, the "hoot" calls of captive male eagle owls (*Bubo bubo*) were stable between years (Lengagne 2001). For free-living mammals, stable calls were reported for a single male blue monkey (*Cercopithecus mitis*—Butynski et al. 1992) and for a single male fallow deer (*Dama dama*—Reby et al. 1998). Our study is the 1st on a free-living mammal where the short- and long-term stabilities of individual features were examined in a representative call sample.

Collecting alarm calls produced toward humans from live traps enabled us to gather repeated alarm calls from the same individuals over a prolonged time period. Humans are commonly used to provoke alarm calling in sciurids (e.g., Koshev and Pandourski 2008; McCowan and Hooper 2002; Slobodchikoff et al. 1991). Factors in our study that could potentially influence the alarm call structure include human individuality (personal dress, movement, etc.) and the height of the grass (visibility). An influence of human dress on alarm call structure has been reported for black-tailed (*Cynomys ludovicianus*) and Gunnison's (*C. gunnisoni*) prairie dogs (Frederiksen and Slobodchikoff 2007; Slobodchikoff et al. 1991). In our study, to minimize the impact of these factors on our results, we limited the number of humans present to 2, and both researchers dressed in camouflage. Although the 1st and 2nd recordings for each individual speckled ground squirrel were always made by the same human collector, variation in alarm call structure did occur between recordings, being stable in some individuals and unstable in others. We did not find any directional trends in individual alarm call characteristics with vegetation growth from the 1st to the 3rd recording, whereas for Gunnison's prairie dogs, the influence of vegetation height has been shown to influence call characteristics (Perla and Slobodchikoff 2002).

Examination of our data indicates that the alarm calls of speckled ground squirrels show high individual distinctiveness within a recording and are consistent with those reported for other sciurid species (Blumstein and Munos 2005; McCowan and Hooper 2002; Nikol'skii and Suchanova 1994). This result suggests that caller's individuality, along with other factors such as predator characteristics, may be responsible for differences in alarm call characteristics observed between different animal samples and between different recordings.

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