Small Mammals Ignore Common Rules: A Comparison of Vocal Repertoires and the Acoustics between Pup and Adult Piebald Shrews Diplomesodon pulchellum

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Abstract

A common rule for mammals vocalizing in the human audible frequency range (20 Hz–20 kHz) suggests that calls are higher in fundamental frequency (f0) in the young than in adults, because of the smaller sound-producing structures of the young. Exclusions are rare, for example the pups of Asian house shrews (Suncus murinus) make some call types of the same or higher pitch than adults. In this study, calls from 62 piebald shrews (Diplomesodon pulchellum), 37 1 to 10-d-old pups from 10 litters and 25 adults were acoustically investigated in captivity. We found eight call types, all within the human audible frequency range: short and long low-frequency squeaks with nearly flat contour, high-frequency squeaks with modulated contour, high-frequency squeaks with fractured contour, short and long screeches, clicks and whimpers. Seven call types were shared by pups and adults, suggesting that this vocal repertoire commences at birth. Against the common rule, the f0 of squeaks was the same in pups and adults, and the f0 of clicks and screeches was even higher in adults than in pups. These results suggest a non-descending ontogenetic pathway that not follows the common physical relationship, of the lower f0 for the larger vocal folds.

Introduction

In mammals whose vocal repertoires are assumed to be fixed at birth, the same call types can be found in pups and adults (Janik & Slater 2000; Seyfarth & Cheney 2010). In mammals that vocalize in the human audible frequency range (20 Hz–20 kHz), the fundamental frequency (f0) is typically higher in pups than in adults (Morton 1977), with minor exclusions (for review, see Matrosova et al. 2007). This is because acoustic differences between young and adults primarily result from the differences in sizes of sound-producing structures (Fitch & Hauser 2002). During ontogeny, the vocal apparatus constantly changes its size and shape, what substantially affects the acoustics (Hammerschmidt et al. 2000; Lapshina et al. 2012). In accordance with the source–filter framework (Fant 1960; Titze 1994; Taylor & Reby 2010), the voice fundamental frequency, generated by ‘source’ in the larynx, is filtered subsequently by the ‘filter’ of the vocal tract, selectively accentuating certain resonance frequencies (formants) and attenuating antiresonances. In the simplest model of mammalian vocal production (uniform tube closed at the end where the sound source is located), the f0 is inversely related to mass and length of the oscillating vocal folds (Fant 1960; Titze 1994; Fitch & Hauser 2002; Taylor & Reby 2010). Exclusions of this common rule are very rare; for instance, in the Asian house shrew (Suncus murinus), the male courtship call arises from the caravanning call of the young, with negligible developmental changes in the acoustics (Schneiderová 2014). Is unknown, however, whether this pathway of vocal development is shared by other species of Soricomorpha.
Similar to the audible calls, ultrasonic calls (greater than the 20 kHz upper limit of the human hearing range) are produced in the larynx by air flow from the lungs. Source-filter theory is equally applicable to audible and ultrasonic calls; however, mechanics for their producing in the larynx are different (Riede 2011). The audible calls are made by passive flow-induced vocal fold oscillations, whereas ultrasonic calls are made by a whistle mechanism, where an acoustic signal is generated because of an obstruction in the path of air jet, such as sharp edge, a hole or a side branch (Riede 2011). The different mechanics for producing audible and ultrasonic calls may explain why mammals calling in ultrasound do not follow the common rule as strictly as those that produce audible calls (Brudzynski et al. 1999; Jin et al. 2011; Arriaga & Jarvis 2013). At the same time, in a few species of mammals, their audible calls also do not fit the common rule, as their f0s are indistinguishable between pups and adults (Matrosova et al. 2007; Swan & Hare 2008; Volodina et al. 2010; Schneiderová 2014).

The interest in vocal behaviour of shrews was stimulated by early findings of ultrasonic echolocation clicks in a few species of shrews (Gould et al. 1964; Forsman & Malmquist 1988; Thomas & Jalili 2004). Other vocalizations aside clicks were also described for a few species of shrews (see review in Schneiderová 2014). Along with tonal calls, many species of shrews make rhythmic calls, with repetitive patterns of short tonal units (Movchan & Shibkov 1982; Siemens et al. 2009; Volodin et al. 2012; Schneiderová 2014). In S. murinus, the ontogenetic shifts of f0 were inconsistent among call types: the f0 was found lower in adults than in pups only in repetitive twitters, whereas in long chirps, it was the same in adults and in pups (Schneiderová 2014). For other shrew species, the ontogeny of time and frequency variables was not investigated so far. To date, pup vocalizations are described only for three species: S. murinus, Blarina brevicauda and Cryptotis parva (Gould 1969; Schneiderová 2014).

Shrews, family Soricomorpha, represent a convenient model for studying vocal ontogeny because of their fast development. At 25–30 d after birth, young shrews are close to adults by body size and body mass (Michalak 1987; Genoud & Vogel 1990). While the ontogenetic studies with shrews would be impossible to conduct with natural populations, captive colonies of a few shrew species allowed collecting data on physical development of many litters (Dryden 1968; Vlasák 1972; Hasler et al. 1977; Michalak 1987). An ontogenetic study of body traits and body mass in 18 litters of piebald shrews demonstrated that they grow very fast (Zaytseva et al. 2013), similarly to other shrews (Dryden 1968; Vlasák 1972; Michalak 1987).

In piebald shrews, weaning is at approximately 20 d of age (Vakhrusheva & Ilchenko 1995) and first copulations were registered at 27 d in females and at 40 d in males (Ilchenko et al. 2011).

In nature, piebald shrews inhabit semi-deserts of Middle Asia and south of Russia, where forage nightly on invertebrates, lizards and spiders on surface or by digging out their prey from depths of 2–3 cm. They cover distances of a few kilometres per night in search of food on the surface of shifting, loose or packed sand (Stalmakova 1949; Dubrovskij et al. 2011). The piebald shrews are solitary, and most non-sexual contacts between two individuals are agonistic or indifferent (Dubrovskij et al. 2011).

Captive adult piebald shrews produce whole-body seismic vibrations, whose rate was the same as the rate of the deep sinusoidal frequency modulation (hereafter ‘pulse rate’) of their loud piercing screech calls (Volodin et al. 2012). No pup vocalizations and no adult vocalizations aside screeches are described for this species. The aim of this cross-sectional study is to compare vocal types and call variables between independent samples of 1 to 10-d-old pups and mature adult piebald shrews. Particular questions we try to answer are (1) whether all call types of pup and adult piebald shrews are audible or some of them expand to the ultrasonic range of frequencies greater than 20 kHz; (2) whether call type sets are shared between pups and adults; and (3) in what extent the values of time, frequency and power variables differ between pups and adults.

Materials and Methods

Study Site and Subjects

Calls were collected from members of a captive colony of piebald shrews at Moscow Zoo, Moscow, Russia, from 1 June to 22 August 2011. Our subjects were 62 piebald shrews, 37 pups (22 males and 15 females from 10 litters, during their first 10 d of life) and 25 adults (10 males and 15 females, older 2 mo). All study animals (3rd–6th generations in captivity) were derived from 27 ancestors, collected in 2008 in the Astrakhan Region, Russia (47°12′33″N 48°18′45″E).

The animals were kept under a natural light regime at room temperature (24–26°C), singly (adults) or in family groups consisting of a mother and littersmates (pups). The animals were housed in plastic cages of 32 × 50 × 40 cm (adults) or in plastic cages of 53 × 76 × 42 cm (family groups), with a bedding...
of sand and dry moss, various shelters and running rings. They received small insectivore chow with insect and mineral supplements and water *ad libitum*.

The 10 study litters, containing in total 37 study pups, originated from 10 different mothers. For each mother, the study litter was her first litter in this breeding season. Before parturition, females were checked twice a day for the appearance of a litter, and birth dates as well as the number of pups were registered. The litter size varied of 3–6 pups (mean ± SD = 3.9 ± 1.2), with 39 pups in total being born. Two pups died at 2 d of age. The date of birth was considered to be the first day of pup life. Study pups were sexed between 3 and 9 d of age based on the presence of nipples in females (Vakhrusheva & Ilchenko 2010). The small size of pups during the study period (the mean body mass of a 5-d-old pup was 2.83 g and body length was 38.5 mm: Zaytseva et al. 2013) prevented individual marking for ethical reasons. The definitive pinna appeared only at 9–10 d (Zaytseva et al. 2013), so newborn piebald shrews could not be individually marked by cuts on ears like Asian house shrews (Schneiderová 2014).

**Call Recording**

All acoustic recordings were conducted in a separate room where no other animals were present, at room temperature 24–26°C during daytime, at the same level of background noise. For sound recordings (sampling rate 96 kHz, 24-bit resolution), we used a Fostex FR-2LE professional digital recorder (Fostex Company, Tokyo, Japan) and a Sennheiser K6-ME64 condenser microphone (Sennheiser electronic, Wedemark, Germany). In addition, to monitor the presence of ultrasound, half of the recordings were made also in the ultrasonic range with sampling rates of 192 kHz or 768 kHz, 16-bit resolution, using a Pettersson D 1000X recorder with built-in microphone (Pettersson Electronik AB, Uppsala, Sweden). All microphones were kept at distance 5–15 cm from the animals, what guaranteed high signal/noise ratio of recordings.

Pup calls were recorded collectively from all pups of each litter (i.e. the calling pups within a litter were not identified individually), with 2-d intervals, in the context of moderate discomfort evoked by separation from the mother on unfamiliar territory, represented by a clean smooth table surface. Each trial lasted 7–14 min and was recorded as a wav file, 41 recording trials in total.

Adult calls (from individually identified animals) were recorded in three contexts: (1) in the context of interaction of two animals on an unfamiliar territory, represented by a clean plastic container 53 × 76 × 42 cm: female–female [2 trials, female–male (6 trials) and male–male (2 trials)], (2) in the context of placing the animal on an elevated disc of 23.5 cm diameter (3 trials), and (3) in the context of taking pups from the nest for recordings (38 trials). Each trial lasted 5–30 min and was recorded as a wav file, 51 recording trials in total.

**Call samples**

Inspection of spectrograms of the acoustic files recorded in the ultrasonic range (frequencies from 20 kHz up to 384 kHz), using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany), did not reveal any ultrasonic components. So, for further acoustic analyses, we used only recordings in audible range of frequencies (from 50 Hz to 24 kHz). For pups, we took 1–5 calls per call type per recording trial, 622 calls in total (Fig. 1). If the number of calls per trial for a particular type was ≤5, all available calls were included into analysis; if it was >5, we randomly selected for analysis 5 calls per call type per trial. For adults, we took randomly 1–10 calls per call type per trial, 484 calls in total (Fig. 1). To decrease pseudoreplication, we selected calls of the same type from different parts of a trial, or when this was impossible, we took calls separated with at least one call.

**Call Analysis**

All spectrographic analyses were performed with Avisoft SASLab Pro and exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). As minimum fundamental frequency f0min of calls always exceeded 1 kHz, before measurements, all wav files were filtered out lower 1 kHz and higher 30 kHz, to remove low-frequency noises and to avoid aliasing (high-frequency aberrations). For all call types, we measured the duration, the maximum amplitude frequency (fpeak), bandwidth (the wide of the frequency peak at a distance –10 dB of amplitude maximum), and the lower (q25), medium (q50) and upper (q75) quartiles, covering, respectively, 25%, 50% and 75% of call energy from the call mean power spectrum, using the option ‘Automatic parameter measurements’ in Avisoft (Fig. 2).

For all call types excluding clicks, we measured the maximum and minimum fundamental frequencies (f0max and f0min) with the reticule cursor in the
spectrogram window (sampling frequency 96 kHz, Hamming window, FFT 1024 points, frame 50%, overlap 96.87%, providing frequency resolution 93 Hz and time resolution 0.3 ms) (Fig. 2). For screeches and short screeches, we additionally measured the mean pulse rate (the mean rate of the deep sinusoidal frequency modulation) as the inverse value of the mean modulation period, using the standard marker cursor in the main window of Avisoft (Fig. 2). For clicks, we additionally measured the click fundamental frequency as the inverse value of the mean f0 period, visible on the strongly extended waveform, using the standard marker cursor in the main window (Fig. 2). This f0 value was used as f0max and as f0min of clicks in subsequent statistic analyses.

Fig. 1: Spectrograms (below) and waveforms (above) of call types emitted by piebald shrews Diplomesodon pulchellum. Each panel presents same type calls of pups (left) and adults (right), demarcated with a vertical line: (a) pup short LF squeaks; (b) adult short LF squeaks; (c) pup long LF squeaks; (d) adult long LF squeaks; (e) pup modulated HF squeaks; (f) adult modulated HF squeaks; (g) pup flat HF squeaks; (h) adult flat HF squeaks; (i) pup screeches; (j) adult screeches; (k) pup short screeches, clicks and whimper; and (l) adult short screeches and clicks. The spectrograms were created with Hamming window, 48 kHz sampling rate, FFT 1024 points, frame 50% and overlap 96.87%. The audio files with these calls are provided in Supplementary Audio S1-S6.
Statistics

Statistical analyses were conducted using STATISTICA (StatSoft, Tulsa, OK, USA) and R v.3.0.1 (R Development Core Team 2009). Means are given as mean ± SD, all tests were two-tailed, and differences were considered significant whenever \( p < 0.05 \). For call types, values were normally distributed for most parameters (Kolmogorov–Smirnov test). As parametrical ANOVA and discriminate function analysis (DFA) are relatively robust to departures from normality (Dillon & Goldstein 1984), this was not an obstacle to the application of these tests.

We used one-way ANOVA with Bonferroni corrections to compare call variables between pups and adults. We used two-factor mixed GLMM, with call type as fixed factor and the personal number of a litter or of an adult animal as random factor, to compare variables of each call type within pups and within adults. Using a standard procedure DFA and a leave-one-out cross-validation DFA (Fischer et al. 2001; Wich et al. 2003; Barros et al. 2011), we calculated the values of call assignment to call type. As the number of calls per call type differed, we used DFA with equal a-priory probabilities of assignment between call types. In each DFA, we used all the eight measured variables and estimated the relative importance of these variables for discrimination using Wilks’ lambda values (Volodin et al. 2014). We used chi-square test to compare the obtained values of correct assignment to age group (pups or adults). To validate DFA results, we calculated the random values of correct assignment to call type by applying randomization procedure with macros, created in R software (R Development Core Team 2009). The random values were averaged from DFAs performed on 1000 randomized permutations on the data sets. Using a distribution obtained by the permutations, we noted whether the observed value exceeded 95% or 99% of the values within the distribution (Solow 1990).

Results

Call Types of Pups and Adults

Based on visual inspection of spectrograms, we subdivided calls by their acoustic structure into eight call types: squeaks (4 types), screeches (2 types), clicks (1 type) and whimpers (1 type) (Fig. 1). Seven of the eight call types were shared by pups and adults, and only one call type (the whimper) was found only in pups. Table 1 presents mean values of measured acoustic variables and ANOVA results for call type comparison between pups and adults.

Short LF (low-frequency) squeaks

Tonal soft calls shorter 100 ms, made since the first day of pup life. The min–max f0 range is 5–10 kHz (Fig. 1a, b). The f0 contour is flat or slightly modulated, what results in call energy concentration within a narrow band. In pups, short LF squeaks attend states of weak arousal at disturbance from littermates or during exploring a new territory. In adults, short LF squeaks attend states of weak arousal at exploring a new territory, non-aggressive contacts with conspecifics, sand digging in proximity of conspecifics and at running in a cage.
### Table 1: Values (mean ± SD) of acoustic variables of eight call types produced by pup and adult piebald shrews, and the results of their comparison between pups and adults with one-way ANOVA.

Designations: N – number of recorded pup litters or adults; n – number of calls; Pup – pups; Ad – adults; f0max – maximum frequency of f0; f0min – minimum frequency of f0; fpeak – maximum amplitude frequency; q25 – lower quartile; q50 – medium quartile; q75 – upper quartile; ANOVA – comparison between pups and adults, and differences considered significant at p < 0.001 after Bonferroni correction are shown in bold.

<table>
<thead>
<tr>
<th>Call type</th>
<th>Age group</th>
<th>N/n</th>
<th>f0max (kHz)</th>
<th>f0min (kHz)</th>
<th>Duration (s)</th>
<th>fpeak (kHz)</th>
<th>Bandwidth (kHz)</th>
<th>q25 (kHz)</th>
<th>q50 (kHz)</th>
<th>q75 (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short LF</td>
<td>Pup 10/169</td>
<td>7.57 ± 0.94</td>
<td>6.4 ± 0.70</td>
<td>0.032 ± 0.017</td>
<td>6.91 ± 0.80</td>
<td>0.81 ± 0.40</td>
<td>6.65 ± 0.89</td>
<td>7.03 ± 0.75</td>
<td>7.59 ± 1.58</td>
<td></td>
</tr>
<tr>
<td>squeak</td>
<td>Ad 18/85</td>
<td>7.79 ± 1.06</td>
<td>6.44 ± 0.76</td>
<td>0.048 ± 0.022</td>
<td>7.37 ± 1.21</td>
<td>0.73 ± 0.39</td>
<td>6.74 ± 1.38</td>
<td>7.80 ± 1.73</td>
<td>9.56 ± 2.97</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>F_{1,187} = 2.63, p = 0.11</td>
<td>0.21, p = 0.64</td>
<td>39.33, p &lt; 0.001</td>
<td>13.02, p &lt; 0.001</td>
<td>2.10, p = 0.15</td>
<td>0.40, p = 0.53</td>
<td>24.39, p &lt; 0.001</td>
<td>47.91, p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long LF</td>
<td>Pup 10/80</td>
<td>8.14 ± 0.77</td>
<td>6.10 ± 0.54</td>
<td>0.122 ± 0.024</td>
<td>7.29 ± 0.79</td>
<td>1.15 ± 0.55</td>
<td>6.95 ± 0.59</td>
<td>7.40 ± 0.67</td>
<td>8.17 ± 1.35</td>
<td></td>
</tr>
<tr>
<td>squeak</td>
<td>Ad 18/59</td>
<td>8.05 ± 1.42</td>
<td>6.17 ± 0.69</td>
<td>0.135 ± 0.035</td>
<td>7.47 ± 1.28</td>
<td>0.77 ± 0.56</td>
<td>6.84 ± 1.56</td>
<td>7.75 ± 1.35</td>
<td>9.94 ± 2.72</td>
<td></td>
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<tr>
<td>ANOVA</td>
<td>F_{1,57} = 0.20, p = 0.65</td>
<td>0.44, p = 0.51</td>
<td>6.61, p = 0.011</td>
<td>1.00, p = 0.32</td>
<td>15.73, p &lt; 0.001</td>
<td>0.32, p = 0.57</td>
<td>4.01, p = 0.047</td>
<td>25.73, p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulated</td>
<td>Pup 10/124</td>
<td>12.18 ± 1.34</td>
<td>7.24 ± 1.21</td>
<td>0.111 ± 0.043</td>
<td>10.23 ± 2.21</td>
<td>1.85 ± 1.19</td>
<td>8.57 ± 1.49</td>
<td>9.94 ± 1.51</td>
<td>11.09 ± 1.50</td>
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<tr>
<td>HF squeak</td>
<td>Ad 20/83</td>
<td>13.45 ± 1.48</td>
<td>7.36 ± 0.86</td>
<td>0.090 ± 0.036</td>
<td>12.11 ± 1.73</td>
<td>1.59 ± 0.76</td>
<td>11.1 ± 1.14</td>
<td>12.24 ± 1.26</td>
<td>13.65 ± 1.81</td>
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<tr>
<td>ANOVA</td>
<td>F_{1,205} = 40.54, p &lt; 0.001</td>
<td>0.63, p = 0.43</td>
<td>17.91, p &lt; 0.001</td>
<td>41.44, p &lt; 0.001</td>
<td>3.18, p = 0.08</td>
<td>165.6, p &lt; 0.001</td>
<td>130.5, p &lt; 0.001</td>
<td>122.1, p &lt; 0.001</td>
<td></td>
<td></td>
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<tr>
<td>Flat HF</td>
<td>Pup 7/50</td>
<td>13.99 ± 1.32</td>
<td>11.34 ± 1.64</td>
<td>0.082 ± 0.037</td>
<td>13.28 ± 1.41</td>
<td>1.27 ± 0.75</td>
<td>12.25 ± 1.57</td>
<td>13.12 ± 1.31</td>
<td>13.54 ± 1.29</td>
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<tr>
<td>squeak</td>
<td>Ad 8/27</td>
<td>14.23 ± 1.81</td>
<td>10.01 ± 1.25</td>
<td>0.110 ± 0.03</td>
<td>12.36 ± 1.27</td>
<td>1.64 ± 0.57</td>
<td>11.87 ± 1.12</td>
<td>12.55 ± 1.25</td>
<td>13.43 ± 1.46</td>
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<td>ANOVA</td>
<td>F_{1,25} = 0.44, p = 0.51</td>
<td>13.60, p &lt; 0.001</td>
<td>12.41, p &lt; 0.001</td>
<td>8.04, p = 0.006</td>
<td>5.13, p = 0.03</td>
<td>1.28, p = 0.26</td>
<td>3.44, p = 0.07</td>
<td>0.12, p = 0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screech</td>
<td>Pup 9/44</td>
<td>10.82 ± 1.27</td>
<td>4.62 ± 1.11</td>
<td>0.121 ± 0.039</td>
<td>10.35 ± 1.86</td>
<td>3.57 ± 1.94</td>
<td>9.10 ± 1.01</td>
<td>11.26 ± 1.24</td>
<td>14.13 ± 1.96</td>
<td></td>
</tr>
<tr>
<td>Ad 25/161</td>
<td>13.21 ± 1.10</td>
<td>6.08 ± 0.79</td>
<td>0.180 ± 0.082</td>
<td>11.62 ± 0.95</td>
<td>2.99 ± 1.76</td>
<td>10.5 ± 7.75</td>
<td>11.86 ± 0.67</td>
<td>13.52 ± 1.20</td>
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<td>ANOVA</td>
<td>F_{1,203} = 151.1, p &lt; 0.001</td>
<td>97.62, p &lt; 0.001</td>
<td>23.44, p &lt; 0.001</td>
<td>41.09, p &lt; 0.001</td>
<td>3.61, p = 0.06</td>
<td>97.54, p &lt; 0.001</td>
<td>18.37, p &lt; 0.001</td>
<td>11.43, p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>Pup 8/13</td>
<td>11.67 ± 1.47</td>
<td>6.09 ± 1.53</td>
<td>0.066 ± 0.020</td>
<td>10.60 ± 1.66</td>
<td>2.27 ± 1.04</td>
<td>9.72 ± 1.06</td>
<td>11.11 ± 1.11</td>
<td>13.08 ± 1.80</td>
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<tr>
<td>screech</td>
<td>Ad 19/38</td>
<td>13.60 ± 1.13</td>
<td>6.67 ± 0.72</td>
<td>0.040 ± 0.020</td>
<td>12.05 ± 1.07</td>
<td>2.19 ± 0.77</td>
<td>11.01 ± 0.72</td>
<td>12.38 ± 0.80</td>
<td>13.93 ± 1.26</td>
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<td>ANOVA</td>
<td>F_{1,49} = 24.13, p &lt; 0.001</td>
<td>3.44, p = 0.07</td>
<td>10.03, p = 0.003</td>
<td>13.19, p &lt; 0.001</td>
<td>0.09, p = 0.77</td>
<td>23.93, p &lt; 0.001</td>
<td>19.82, p &lt; 0.001</td>
<td>3.56, p = 0.07</td>
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<tr>
<td>Click</td>
<td>Pup 10/128</td>
<td>8.43 ± 2.29</td>
<td>8.43 ± 2.29</td>
<td>0.004 ± 0.001</td>
<td>7.63 ± 2.82</td>
<td>6.02 ± 2.30</td>
<td>6.15 ± 1.96</td>
<td>8.04 ± 2.15</td>
<td>9.98 ± 2.43</td>
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<tr>
<td>Ad 5/31</td>
<td>8.39 ± 1.72</td>
<td>6.62 ± 1.12</td>
<td>0.004 ± 0.001</td>
<td>9.26 ± 1.89</td>
<td>5.18 ± 1.59</td>
<td>7.94 ± 1.47</td>
<td>9.61 ± 1.67</td>
<td>11.56 ± 1.75</td>
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<tr>
<td>ANOVA</td>
<td>F_{1,57} = 7.88, p = 0.006</td>
<td>7.88, p = 0.006</td>
<td>0.03, p = 0.87</td>
<td>9.36, p = 0.003</td>
<td>5.51, p = 0.02</td>
<td>22.41, p &lt; 0.001</td>
<td>14.24, p &lt; 0.001</td>
<td>11.60, p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whimper</td>
<td>Pup 3/14</td>
<td>3.07 ± 0.73</td>
<td>1.81 ± 0.47</td>
<td>0.029 ± 0.015</td>
<td>2.28 ± 0.57</td>
<td>1.20 ± 1.34</td>
<td>2.75 ± 0.68</td>
<td>5.30 ± 1.18</td>
<td>7.85 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>Ad no</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>
Long LF (low-frequency) squeaks
Tonal soft calls, made since the first day of pup life. The duration varies from 100 to over 200 ms (Fig. 1c, d). The min–max f0 range is 5–10 kHz. The f0 contour is flat or slightly modulated; call energy is concentrated within a narrow frequency band. In pups, long LF squeaks attend states of weak arousal at disturbance from littermates or during exploring a new territory and represent the most usual contact calls with littermates or a mother. In pups older 10 d, these calls attend peaceful interactions and precede successful copulations.

Modulated HF (high-frequency) squeaks
Tonal calls of moderate intensity, made since the first day of pup life (Fig. 1e, f). The duration varies from 40 to 200 ms. The min–max f0 range is 6–15 kHz. The f0 contour is deeply modulated, with a smooth increase of f0 to maximum followed with smooth decrease to the end of a call, sometimes with a plateau around f0max. In some calls, the f0 increases or decreases rapidly. In pups, modulated HF squeaks attend states of weak arousal during peaceful contacts with littermates. In adults, they attend both peaceful and aggressive interactions with conspecifics, for example when a non-receptive female is rejecting a courting male.

Flat HF (high-frequency) squeaks
Tonal calls of high intensity, made since the first day of pup life (Fig. 1g, h). This is most intensive of all types of squeaks. The duration varies from 60 to 150 ms. The min–max f0 range is 10–16 kHz. The f0 is kept high throughout the duration of a call. A peculiar characteristic of this call type is the fractured contour with sudden falls and rises of f0. The fpeak is the highest compared to all other call types. In pups, flat HF squeaks attend the states of elevated arousal at disturbance from littermates or during experimental translocations. In adults, they attend aggressive interactions, for example when a non-receptive female aggressively chases a male.

Screeches
Tonal calls of high intensity with a very deep sinusoidal modulation, made since the third day of pup life (Fig. 1i, j). The duration is 60–200 ms in pups and over 400 ms in adults. The very low f0min (2.5–6 kHz) along with the very high f0max (10–15 kHz) together create the wideband frequency spectrum. The modulation period of f0 is kept stable throughout a call or increases slightly to the end of a call. The modulation period of f0 is much longer in pups (10–16 ms) than in adults (5.5–8 ms). These modulation periods correspond, respectively, to pulse rates of 60–108 Hz in pups (91.48 ± 13.56 Hz) and of 128–185 Hz in adults (158.31 ± 12.33 Hz). Some screeches display descending modulation of the overall contour, thus reminding a modulated squeak superimposed with sinusoid. Screeches are most loud and remarkable calls of piebald shrews. Their function is warning rather than threatening. In either pups or adults, they attend states of anxiety in situations of grasping and fixation of animals in human hands, during contacts with unfamiliar conspecifics and during self-defending or defending of a shelter against conspecifics or against an approaching human hand.

Short screeches
Tonal calls of high intensity, made since the third day of life (Fig. 1k, l). They are very similar to screeches, although much shorter (below 60–100 ms), because they contain only 2–4 periods of the deep sinusoidal modulation of f0. The variation of pulse rates of short screeches is comparable to those of screeches and varies of 40–100 Hz (82.12 ± 18.17 Hz) in pups and of 107–165 Hz (134.76 ± 15.24 Hz) in adults. Short screeches often have a terminal squeaky part that can exceed by duration the screechy part. Short screeches attend the same situations as screeches.

Clicks
Short sound pulses with a wideband spectrum, made since the first day of life (Fig. 1k, l). Clicks envelope the range of frequencies from nearly zero to 20–30 kHz, so their bandwidth is the widest among all call types. The fpeak varies from 3 to 14 kHz. A click waveform contains 3–4 periods of f0, fading by amplitude to the end of a click; their values robustly coincide with the value of fpeak (Fig. 2). In pups, clicks attend states of weak arousal when a caller is on unfamiliar territory or at tactile contacts with littermates. However, we did not find strict relations between making clicks and specific situations, actions or
movements. In adults, clicks were recorded only from five individuals, in contexts of elevated disc exploring and during male–female pairing. The low intensity of this call allows to reliably distinguishing it from strikes over table surface only when the level of background noise is low.

Whimper

Whimpers represent low-frequency tonal calls with a harmonically rich spectrum, often starting with a very short wideband fragment (Fig. 1k). These calls were found only in pups from 2nd to 6th day of pup life inclusive. The duration is 30–50 ms. The f0 of whimpers is the lowest among all call types, with f0max not exceeding 4 kHz. The f0 smoothly decreases from the start to the end of a call. The f0min varies between 1.0 and 2.5 kHz. These calls occur very rarely, and their relation to particular situations is unclear.

Call Variables of Pups and Adults

With one-way ANOVA, we compared the acoustic variables of pups and adults for each call type. Short LF squeaks of pups and adults did not differ by f0max and f0min, and were significantly longer in adults than in pups (Table 1). The fpeak, q50 and q75 were significantly higher in adults than in pups, for the account of the energy shift towards higher frequencies in adults. Long LF squeaks of pups and adults did not differ by f0max, f0min and duration (Table 1). Unlike short LF squeaks, the energy shift towards higher frequencies in adults resulted in their significantly higher q75, but not q50 and fpeak compared to pups. Modulated HF squeaks were significantly higher in adults than in pups by f0max but not by f0min and were significantly shorter in adults than in pups (Table 1). The energy shift towards higher frequencies in adults resulted in significantly higher fpeak and all the three quartiles did not differ between pups and adults. Screeches were significantly higher by f0max and f0min and significantly longer in adults than in pups (Table 1). As fpeak, q25 and q50 were significantly higher, the q75 was significantly lower in adults than in pups; so, call energy was concentrated in approximately the same parts of call spectra in either pups or adults. The pulse rate was significantly higher in screeches of adults compared to screeches of pups. In short screeches, differences between pups and adults were the same as in screeches, with the exception of duration, which was found significantly shorter in adults than in pups (Table 1). As in screeches, the pulse rate of short screeches was significantly higher in adults than in pups.

Clicks were significantly higher in adults than in pups by f0, but did not differ between pups and adults by duration (Table 1). For the account of the noticeable energy shift towards higher frequencies in adults compared to pups, the fpeak and all the three quartiles were significantly higher in adults than in pups.

DFAs For Call Type: Pups, Adults and Both

GLMM showed significant effect of call type on all the eight measured call variables of pups and adults (Table 2). So, we included all the eight variables in subsequent DFAs. We used three different DFAs. Two of the DFAs (one for pups and one for adults) were conducted to test whether call types were acoustically distinct. The third DFA (run on a pooled call sample of calls of pups and adults) was conducted to test the continuity of vocal repertoire through ages.

A DFA, classifying calls of pups to 8 types, correctly assigned 90.7% of the calls (Table 3). One-leave-out cross-validation did not show a decrease of correct assignment to call type (90.2%); the value of correct assignment was significantly higher (p < 0.001) than

<table>
<thead>
<tr>
<th>Age group</th>
<th>f0max</th>
<th>f0min</th>
<th>Duration</th>
<th>fpeak</th>
<th>bandwidth</th>
<th>q25</th>
<th>q50</th>
<th>q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pup</td>
<td>F7,605 = 241.2</td>
<td>F7,605 = 146.9</td>
<td>F7,605 = 255.0</td>
<td>F7,605 = 115.6</td>
<td>F7,605 = 201.9</td>
<td>F7,605 = 156.1</td>
<td>F7,605 = 169.0</td>
<td>F7,605 = 140.9</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ad</td>
<td>F6,458 = 329.1</td>
<td>F6,458 = 130.2</td>
<td>F6,458 = 118.8</td>
<td>F6,458 = 208.8</td>
<td>F6,458 = 42.1</td>
<td>F6,458 = 223.1</td>
<td>F6,458 = 204.6</td>
<td>F6,458 = 60.4</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>
the random value (15.5 ± 1.3%). The value of correct classification to call type varied from 100% for long LF squeaks and whimpers to 69.2% for short screeches. In order of decreasing importance, the f0min, f0max, duration and bandwidth were mainly responsible for discrimination (Table 4).

A DFA, classifying calls of adults to 7 types, assigned correctly 82.6% of the calls (Table 3). One-leave-out cross-validation did not show a decrease of correct assignment to call type (81.0%). The value of correct assignment was significantly higher (p < 0.001) than the random value (16.4 ± 1.1%), but was significantly lower than in the DFA for calls of pups (χ2 = 14.98, df = 1, p < 0.001). The value of correct classification to call type varied from 96.8% for clicks to 63.9% for modulated HF squeaks. The variables mainly responsible for discrimination (f0min, f0max, duration and bandwidth) were the same as in the DFA for calls of pups and in the DFA for calls of adults (Table 4).

A DFA, classifying a pooled sample of calls from pups and adults to 7 call types (excluding whimper, presented only in pups), assigned correctly 83.7% of the calls (Table 3). One-leave-out cross-validation did not show a decrease of correct assignment to call type (83.1%); the value of correct assignment was significantly higher (p < 0.001) than the random value (16.5 ± 0.8%). This value did not differ significantly from those obtained in the DFA for adults (χ2 = 0.20, df = 1, p = 0.66), but was significantly lower than those obtained in the DFA for pups (χ2 = 15.66, df = 1, p < 0.001). The variables mainly responsible for discrimination (f0min, f0max, duration and bandwidth) were the same as in the DFA for calls of pups and in the DFA for calls of adults (Table 4).

**Discussion**

We found eight call types in the vocal repertoire of piebald shrews. Seven of them, excluding whimpers, were shared by pups and adults. DFAs for call type, conducted separately for pups and adults, supported this classification. The DFA for call type, conducted

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### Table 3: DFA results for call assignment to correct type for pups, for adults and for a pooled sample of calls from pups and adults. Designations: n – number of calls; Pup – pups; Ad – adults; Pup+Ad – the pooled sample of calls from pups and adults

<table>
<thead>
<tr>
<th>Call type</th>
<th>Pup</th>
<th>Ad</th>
<th>Pup+Ad</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Correct assignment score (%)</td>
<td>n</td>
</tr>
<tr>
<td>Short LF squeak</td>
<td>169</td>
<td>95.3</td>
<td>85</td>
</tr>
<tr>
<td>Long LF squeak</td>
<td>80</td>
<td>100.0</td>
<td>59</td>
</tr>
<tr>
<td>Modulated HF squeak</td>
<td>124</td>
<td>83.1</td>
<td>83</td>
</tr>
<tr>
<td>Flat HF squeak</td>
<td>50</td>
<td>92.0</td>
<td>27</td>
</tr>
<tr>
<td>Screech</td>
<td>44</td>
<td>70.5</td>
<td>161</td>
</tr>
<tr>
<td>Short screech</td>
<td>13</td>
<td>69.2</td>
<td>38</td>
</tr>
<tr>
<td>Click</td>
<td>128</td>
<td>93.8</td>
<td>31</td>
</tr>
<tr>
<td>Whimper</td>
<td>14</td>
<td>100.0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>622</td>
<td>90.7</td>
<td>484</td>
</tr>
</tbody>
</table>

---

### Table 4: Wilks’ Lambda values and variable effect for each acoustic variable included into the three independent DFAs for call assignment to correct type for pups (Pup), for adults (Ad) and for the pooled sample of calls of pups and adults (Pup+Ad). The smaller the Wilks’ Lambda value, the greater the contribution of the given call variable to the overall discrimination. For each DFA, the four variables, mostly contributed into discrimination, are given in bold.

<table>
<thead>
<tr>
<th>Call variable</th>
<th>Pup</th>
<th>Ad</th>
<th>Pup+Ad</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wilks’ Lambda</td>
<td>Variable effect</td>
<td>Wilks’ Lambda</td>
</tr>
<tr>
<td>f0max</td>
<td>0.450238</td>
<td>F = 105.9</td>
<td>0.468607</td>
</tr>
<tr>
<td>f0min</td>
<td>0.337605</td>
<td>F = 170.1</td>
<td>0.337382</td>
</tr>
<tr>
<td>Duration</td>
<td>0.487464</td>
<td>F = 91.2</td>
<td>0.488383</td>
</tr>
<tr>
<td>Fpeak</td>
<td>0.875034</td>
<td>F = 13.2</td>
<td>0.935283</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>0.507821</td>
<td>F = 84.0</td>
<td>0.547031</td>
</tr>
<tr>
<td>q25</td>
<td>0.805103</td>
<td>F = 21.0</td>
<td>0.903862</td>
</tr>
<tr>
<td>q50</td>
<td>0.765174</td>
<td>F = 26.6</td>
<td>0.876793</td>
</tr>
<tr>
<td>q75</td>
<td>0.922174</td>
<td>F = 7.3</td>
<td>0.925791</td>
</tr>
</tbody>
</table>
with the pooled sample of calls from pups and adults, confirmed a strong similarity of call types in both age groups. Therefore, the vocal repertoire of the piebald shrew displays a continuity of call type from pups to adults. This is distinctive from call type ontogeny in Asian house shrews, where call types were found appearing and disappearing throughout ontogenesis (Schneiderová 2014). Of seven call types that were reported in the Asian house shrew pups, only 5 call types were presented also in adults, whereas two other call types disappeared and 10 new call types appeared at maturation (Schneiderová 2014).

Unlike Asian house shrews, the vocal ontogeny of piebald shrews displays some changes of structural variables rather than the changes of call type sets. These changes in the acoustic variables between pup and adult piebald shrews were either minor or significant depending on call type. In three of the seven shared call types, the duration was significantly higher in adults than in pups; in two other call types, it was significantly higher in pups than in adults; and in two call types (long LF squeaks and clicks), it was indistinguishable between pups and adults. In all call types besides flat HF squeaks, the call spectral energy was noticeably shifted towards higher frequencies in adults compared to pups. In all types of squeaks, the F0 of pup calls was indistinguishable from those of adults, whereas in screeches and clicks, it was even significantly lower in pups than in adults.

Therefore, piebald shrews belong to mammalian taxa that do not display an ontogenetic decrease of F0 from pups to adults. This is one extra exclusion of the common rule for mammals (Morton 1977; Matrosova et al. 2007) suggesting the lower F0 in adults than in the young due to the larger larynx of adults (Titze 1994; Fitch & Hauser 2002). For insectivores, similar findings of indistinguishable F0s between pups and adults were reported for the Asian house shrew male courtship calls, which arise from caravanning calls of the young of this species, with negligible changes in F0 and in other acoustics along development (Schneiderová 2014). In rodents, similar findings of indistinguishable F0s were reported for alarm calls of juvenile and adult ground squirrels: speckled Spermophilus suslicus, yellow S. fulvus (Matrosova et al. 2007; Volodina et al. 2010) and Richardson’s S. richardsonii (Swan & Hare 2008). Consistently, playback-based studies revealed that adult Richardson’s ground squirrels cannot discriminate between alarm calls of juveniles and adults (Swan & Hare 2008). In small mammals that vocalize in the ultrasonic range of frequencies, the reported developmental pathways varied across call type and species. Overall, calls of adults were reported lower in frequency than in pups in domestic mice Mus musculus (Grimsley et al. 2011; Arriaga & Jarvis 2013) and were reported higher in frequency in adults than in pups in leaf-nosed bats Hipposideros pomona (Jin et al. 2011). In Norway rats Rattus norvegicus, the initial very broad range of frequencies of pups was further split in adults into three non-overlapping frequency ranges of 1–10, 22 and 50 kHz calls (Brudzynski et al. 1999; Brudzynski 2005; Riede 2011; Riede et al. 2011). However, the pathways of F0 ontogeny of ultrasonic and audible calls are not immediately comparable, because mechanics for their producing may differ even within species, for example in the Norway rat (Riede 2011).

In the young and adults of speckled and yellow ground squirrels, the sizes of their larynges were proportional to the condylobasal length of the skull (Matrosova et al. 2007). So, the similarity of F0 between the young and adult ground squirrels does not result from the accelerated growth of the larynx relatively to the growth of the body of the young. In mammals, pups have larger heads relative to body size compared to their adults, but their absolute sizes are substantially different. For instance, in piebald shrews, the head length of a 1-d-old pup comprises only 48.6% of the adult head length (Zaytseva et al. 2013). Explaining hypothesis for the indistinguishable F0s between pups and adults in piebald shrews and some other species comes from different mechanics of very small vocal folds compared to those of larger-sized mammals (Riede et al. 2011).

In both short and long screeches of piebald shrews, the pulse rate was significantly higher in adults than in pups, probably for the account of better control on the work of the vocal apparatus in adults. The pulse rate might reflect the development of a unique for mammals’ ability of seismic whole-body vibration, directly observed in adult and subadult piebald shrews (Volodin et al. 2012). Although the relation between seismic vibration and superfascia sinusoidal modulation of screeches was not yet convincingly confirmed, a previous study has demonstrated a perfect coincidence of the pulse rate of screeches with the rate of seismic vibrations in the same individual piebald shrews (Volodin et al. 2012). The screech-like calls were reported for adults in many species of shrews (Movchan & Shibkov 1982; Konstantinov & Movchan 1985), but the ontogeny of the acoustics of these calls was studied only for a single litter of Laxmann’s shrews Sorex cascatiens. Similarly to piebald shrews, the rate of sinusoidal modulation of twitters of Laxmann’s shrews was significantly higher in adults (212 Hz) than in pups (78 Hz at 9 d of age), with a

No ultrasound was found in our study in either pup or adult piebald shrews, and all registered calls and clicks were produced in the human audible frequency range. Accordingly, no ultrasonic clicks were found in this study, and only clicks not exceeding 15 kHz were found in either pups or adults. These data are consistent to our previous study, where we also tried to find any ultrasonic signals in adult and subadult piebald shrews (Volodin et al. 2012). The audible clicks of piebald shrews differ from ultrasonic orientation clicks, reported for a few other species of shrews (Gould et al. 1964; Gould 1969; Buchler 1976; Tomasi 1979; Forsman & Malmquist 1988; Thomas & Jalili 2004). In piebald shrews in this study, clicks were mostly produced by the youngest pups, which were still helpless, blind and with closed ears. We suppose that piebald shrew pups might produce their audible clicks for orientation, and that the low frequency of these impulses might be related to the closed acoustic ducts and the lack of pinnae. Another explaining hypothesis is that the clicks could serve for attracting mother’s attention by pups that were out of the nest and experienced hypothermia (e.g. Hofer 1996). In adult piebald shrews, the audible clicks were registered very rarely and only in animals that were placed on an elevated disc. It is possible, however, that adult animals on the ground bedding or leaf litter cannot use their soft clicks for orientation, because they are unable to discriminate them from overlapping mechanical noises (e.g. paw and claw strikes and rustles). Recent experiments demonstrated that for orientation in the bedding, shrews can use their much louder twitters (probably analogous to screeches of piebald shrews) rather than soft clicks (Siemers et al. 2009).

In conclusion, our results contribute to general understanding of variation on pathways of vocal ontogeny and flexibility of vocalisations in mammals. We found that piebald shrews, producing only audible calls, demonstrate a rather unusual pathway of f0 ontogeny, with the same or lower f0 in pups compared to adults, in all types of tonal calls. Usually, the pathway of vocal ontogeny of audible calls shows a steady descent of f0 (e.g. Briefer & McElligott 2011; Efremova et al. 2011) or, exclusively in human males, displays ‘voice breaking’, with an abrupt fall of f0 at puberty (Lee et al. 1999). Previously, in mammals vocalizing in the audible frequency range, a similar to piebald shrews pattern of vocal ontogeny was reported only for the caravanning call of one of shrew species (Schneiderová 2014) and for alarm calls of a few species of ground squirrels (Matrosova et al. 2007; Swan & Hare 2008; Volodin et al. 2010). Further longitudinal study should reveal relations between vocal and body development of piebald shrews across ages.

Acknowledgements

We thank the staff of Moscow Zoo for their help and support. We are sincerely grateful to O.A. Filatova for her help with statistics. We are sincerely grateful to the two anonymous reviewers for reviewing the paper and valuable comments. During our work, we adhered to the ‘Guidelines for the treatment of animals in behavioural research and teaching’ (Anim. Behav., 2006, 71, 245–253) and to the laws of the Russian Federation, where the research was conducted. This study was approved by the Committee of Bio-ethics of Lomonosov Moscow State University (research protocol # 2011-36). No animal has suffered somehow due to the data collection. Data collection and treatment were supported by the Russian Foundation for Basic Research Grant No 12-04-00260a (for IV, AZ and EV). Conducting further analyses for preparing revised versions of the manuscript was supported by the Russian Scientific Foundation, Grant No 14-14-00237 (for IV, AZ and EV).

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Supporting Information

Additional supporting information may be found in the online version of this article:

Audio S1: Sound file of short LF squeaks of piebald shrews, 4 from pups and 4 from adults.

Audio S2: Sound file of long LF squeaks of piebald shrews, 2 from pups and 2 from adults.

Audio S3: Sound file of modulated HF squeaks of piebald shrews, 2 from pups and 3 from adults.

Audio S4: Sound file of flat HF squeaks of piebald shrews, 4 from pups and 3 from adults.

Audio S5: Sound file of screeches of piebald shrews, 2 from pups and 2 from adults.

Audio S6: Sound file of short screeches, clicks and whimpers of piebald shrews, 2 short screeches, 2 clicks and 1 whimper from pups and 2 short screeches and 2 clicks from adults.