

Non-individualistic ultrasonic and audible isolation calls throughout ontogeny in a rodent, *Eolagurus luteus*

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ABSTRACT

Acoustic individuality is present in diverse taxa of mammals and birds, becoming especially prominent in those age groups for which discriminating conspecifics by voice is critically important. This study compares, for the first time, the ontogenetic changes of acoustic individuality of ultrasonic and audible calls (USVs and AUDs) across 12 age-classes (from neonates to adults) in captive yellow steppe lemmings *Eolagurus luteus*. We found that, in this rodent species, the isolation-induced USVs and AUDs are not individually distinct at any age. We discuss that this result is unusual, because discriminating individuals by individualistic vocal traits may be important for such a social species as yellow steppe lemming. We also discuss the potential role of acoustic individuality in studies including rodent models.

1. Introduction

Individualistic vocal traits are found in diverse mammalian and bird taxa (e.g., Terry et al., 2005; Carlson et al., 2020). In mammals, presence of individualistic vocal traits is found e.g., in primates (Lau et al., 2020); artiodactyls (Sibiryakova et al., 2018; Green et al., 2019), lagomorphs (Volodin et al., 2018, 2021a) and in rodents (Matrosova et al., 2011; Ancillotto and Russo, 2016; Verzola-Olivio et al., 2021). Among mammals, non-individualistic calls are rare exclusions. For instance, non-individualistic pup nursing calls were found in Hawaiian monk seals *Monachus schauinslandi* (Job et al., 1995). Non-individualistic rutting calls were also reported in male fallow deer *Dama dama* (Briefer et al., 2010).

Individualistic calls serve for maintaining parent-offspring relationship (Klenova et al., 2009; Sèbe et al., 2011; Sibiryakova et al., 2015;

Volodin et al., 2019b), for maintaining group cohesion (Owren and Rendall, 1997, 2001) and for facilitating mate recognition (Klenova et al., 2011; Curé et al., 2016). Reliability of individual vocal signature depends on call type (Volodin et al., 2011) and can change within season (Matrosova et al., 2009), between years (Smirnova et al., 2016; Matrosova et al., 2010; Schneiderová et al., 2017) and along development (Klenova et al., 2009; Lapshina et al. 2012; Favaro et al., 2014). The most powerful factor affecting the developmental changes of acoustic individuality might be the ontogenetic changes of vocal morphology (Lungova et al., 2015; Volodin et al., 2017a; Riede et al., 2020). Functional explanations, such as different functions of vocalizations in young and adults (Lau et al., 2020) or varying needs in parental care along ontogeny (Klenova et al., 2009) are also relevant for variation of acoustic individuality along ontogeny. For instance, in crane chicks, the reliability of individual vocal signature is moderate on the parental

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territory, but increases enormously in adolescents in large flocks where they strongly need parental defense, and then decreases again in independent young adults (Klenova et al., 2009).

In most rodents, acoustic communication between parents and offspring or between adults is primarily based on the ultrasonic calls over 20 kHz (Vieira and Brown, 2002; Riede, 2011, 2013, 2018; Brudzynski, 2014; Pasch et al., 2017; Klenova et al., 2021b) or on the audible-through-ultrasonic calls, starting below and ending above 20 kHz or vice versa (Pasch et al., 2011; Kobayasi and Riquimaroux, 2012; Campbell et al., 2014; Riede and Pasch, 2020). At the same time, pups of precocious caviomorph rodents seem to vocalize only in the audible range of frequencies (Tokumaru et al., 2004; Long, 2007; Monticelli and Ades, 2013), although Long (2009) interprets the upper harmonics of the audible call as ultrasound. Some rodents, as yellow steppe lemmings *Eolagurus luteus*, equally use at isolation and handling both ultrasonic and audible call types from the first day onwards throughout ontogeny (Rutovskaya, 2019; Yurlova et al., 2020; Klenova et al., 2021a; Volodin et al., 2021b).

Among mammals which do not experience production vocal learning (Janik and Slater, 1997, 2000) and have innate vocal repertoires, the ontogeny of acoustic individuality only was studied in audible contact calls of goitred gazelles *Gazella subgutturosa* (Lapshina et al., 2012) and in audible contact calls of Guinea pigs *Cavia porcellus* (Baklová, 2016). There are no studies comparing the ontogeny of acoustic individuality in ultrasonic (USV) and audible (AUD) vocalizations in the same context within species. For USVs of adult rodents, a presence of individual vocal signature only was reported for the courtship vocalizations of male domestic mice *Mus musculus* (Hoffmann et al., 2012; Marconi et al., 2020). For USVs of pup rodents, individuality of isolation-induced USVs was only investigated regarding temporal traits (Barnes et al., 2017) and call rate (Verjat et al., 2019) in domestic mice. Individualistic AUDs in the social context were found in adult capybaras *Hydrochoerus hydrochaeris* (Lacerda et al., 2014). Individualistic AUDs in the isolation and handling contexts were found in adult northern birch mice *Sicista betulina* (Volodin et al., 2019a) and in infant Guinea pigs (Tokumaru et al., 2004).

The yellow steppe lemming is a steppe-dwelling Asian rodent (Bannikova et al., 2019), with adult body mass about 100 g on average in either sex in captivity (Yurlova et al., 2020). This is a diurnal social species living in family groups with a few subsequent litters of different ages (Smorkatcheva et al., 2008, 2009; Yurlova et al., 2020). After maturation, the offspring often stay in the family group but do not breed due to effective breeding regulation mechanism (Smorkatcheva et al., 2008, 2009) which probably involves vocalization. Whereas the ontogeny of the acoustic variables of isolation-induced USVs and AUDs is studied in detail in yellow steppe lemming (Yurlova et al., 2020; Volodin et al., 2021b), the ontogeny of acoustic individuality in these calls has yet to be studied in this species.

Yellow steppe lemmings display very rapid physical pup development and early formation of adult-like vocal repertoires of USVs and AUDs (Yurlova et al., 2020; Volodin et al., 2021b). The range of USVs in yellow steppe lemmings is from 25.7 to 52.9 kHz (Yurlova et al., 2020), whereas the range of AUDs is from 0.67 to 3.48 kHz (Volodin et al., 2021b). Already since the age of 9–12 days, pups produce USVs and AUDs which are practically undistinguishable by their acoustic characteristics from the same call types of adults. The same acoustic variables can be measured in USVs and AUDs of yellow steppe lemmings throughout ontogeny (Yurlova et al., 2020; Volodin et al., 2021b). Taken together, these characteristics make yellow steppe lemming a convenient model for comparing the changes of acoustic individuality in USVs and AUDs throughout ontogeny.

In this study, we expect that the degree of individualization of both USVs and AUDs will increase from neonates through adolescents to adults. We suppose, that for neonate pup rodents, individualistic isolation-induced USVs and AUDs are unimportant, as pups just need from their parents a solution of their problems (e.g., to warm, to feed, to

return a pup to the nest). For rodent parents, pup vocal identity is also expected to be unimportant, as all pups around the nest are their own, so it makes no difference which particular individual is calling. At the same time, for adult and adolescent lemmings, individualistic USVs and AUDs might be important, as they can be used in social contexts other than isolation and discomfort (at aggression, peaceful contacts in family groups, maintaining dyad relationships, mating behavior). The aim of this study was to investigate the ontogeny of acoustic individuality in USVs and AUDs across 12 age-classes (from neonates to adults) in captive yellow steppe lemmings.

2. Material and methods

2.1. Study site and animals

Audio recordings were made from 162 captive-born individual yellow steppe lemmings at different ages from neonates to breeding adults at Moscow Zoo, Moscow, Russia, in February–July 2018 and March–April 2020 during experimental isolation and handling procedure. The experimental adults were breeding parents of family groups. Pups at the age until 20–30 d, used in the experiments, were kept in family groups with their parents. The older pups (from 20 to 60 d) were kept with their parents, sometimes in a group could present pups of the next younger litter. Adolescents which did not participate in the experiments were separated from the parents at 30–60 d of age.

The animals were kept under a natural light regime at room temperature (22–25 °C), in family groups consisting of two parents and littermates of 1–3 subsequent litters, in wire-and-glass cages of 50×100×35 cm, with a bedding of sawdust and hay and various shelters. They received custom-made small desert rodent chow with mineral supplements and fruits and vegetables ad libitum as a source of water (for housing details, see Yurlova et al., 2020). The adults were individually marked, whereas the small size of pups prevented individual marking for ethical reasons until 20–25 d of age. Pups were sexed after 20–25 d of age based on visible testicles in males or vagina in females.

For selecting individuals for call-eliciting trials, we used a cross-sectional approach, with 12 non-overlapping age classes, for avoiding the effects of the repeated testing on development of the experimental pups (Yurlova et al., 2020; Volodin et al., 2021b). With this approach, each individual was tested only once, at one of 12 age classes (1–4 d, 5–8 d, 9–12 d, 13–16 d, 17–20 d, 21–24 d, 25–28 d, 29–32 d, 33–36 d, 37–40 d; 41–60 d and adults over 90 d). The day of birth was considered zero day of individual life. We could not use the longitudinal approach with the same individuals repeatedly tested in each age class, because, in this species, a regular handling may suppress body growth (Yurlova et al., 2020).

2.2. Call-eliciting trials

Experimental trials for eliciting calls (both USVs and AUDs) were conducted in a separate room at 22–25 °C during daytime. The trial and audio recording started, when the focal animal was placed to the experimental setup, either in a clean plastic hutch (190×130×70 mm for 1–12 d pups) or in a plastic cylinder without bottom (diameter 193 mm, high 170 mm for 13–60 d pups and adults), placed on even plastic table surface. Both the plastic huge and cylinder were open from above, i.e., from the side where the microphone was placed.

Each trial had four stages: isolation (120 s); touch (120 s), handling (120 s) and measurement (120 s). All animals were tested singly. During the isolation stage, the focal animal was just isolated in an experimental setup. During the touch stage, the experimenter (DDY or IAV) gently touched the focal animal with a cotton bud, approximately twice per second. During the handling stage, the experimenter accurately grasped the animal and kept with belly up. During the measurement stage, the experimenter measured body length, head length, foot length and tail length with an electronic caliper, continuing keeping animal in hands.

The end of measurements was the end of the trial (for details of the call-eliciting procedure, see Volodin et al., 2021b). The measured parameters of body size were used in other studies (Yurlova et al., 2020; Volodin et al., 2021b).

2.3. Call recording

For recording USVs at sampling rate 384 kHz and 16 bit resolution (see Yurlova et al., 2020 for details), we used a Pettersson D1000X recorder with built-in microphone (Pettersson Elektronik AB, Uppsala, Sweden). For recording the AUDs at sampling rate 48 kHz and 16 bit resolution (see Volodin et al., 2021b for details), we used a solid state recorder Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with a Sennheiser K6-ME66 cardioid electret condenser microphone (Sennheiser electronic, Wedemark, Germany). Both microphones (for USV and AUD recordings) were established stationary at distance 35 cm above the animal. Recording of each trial was stored as two wav-files, one ultrasonic and one audible.

2.4. Samples of animals and calls

Of the 162 subject animals which provided calls for this study, 78 individuals were the same for USV and AUD samples, whereas among other 84 individuals, 42 were only used for creating USV sample and other 42 only used for creating AUD sample. Samples of animals did not entirely overlap, because some animals produced more high-quality USVs whereas some others produced more high-quality AUDs.

Call samples for acoustic measurements were created using visual inspection of spectrograms of acoustic files with Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). All selected calls were of high sound-to-noise ratio and without superimposed noise. Contour shape and presence of nonlinear phenomena were not taken into account at call selection for analysis (Yurlova et al., 2020; Volodin et al., 2021b).

Call samples for acoustic measurements were created using visual inspection of spectrograms of acoustic files with Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). All selected calls were of high sound-to-noise ratio and without superimposed noise. Contour shape and presence of nonlinear phenomena were not taken into account at call selection for analysis (Yurlova et al., 2020; Volodin et al., 2021b).

A sample of 1200 USVs included 10 ultrasonic calls per individual, 120 individuals, 10 individuals per age class. This sample included 1170 USVs from 117 individuals used in Yurlova et al. (2020) and 30 USVs from 3 individuals added in this study. We took USVs randomly from different parts of the isolation stage of each subject individual (see Yurlova et al., 2020 for details). A sample of 1200 AUDs included 10 audible calls per individual, 120 individuals, 10 individuals per age class. The sample of 1200 AUDs was the same as in the study by Volodin et al. (2021b). We took AUDs randomly from different parts of the handling and measurement stages from each subject individual (see Volodin et al., 2021b for details).

2.5. Acoustic analysis

The same 6 acoustic variables were measured in both USVs and AUDs. For measuring USVs, we used the following settings: sampling frequency 384 kHz, Hamming window, Fast Fourier Transform 1024 points, frame 50%, overlap 87.5%, providing frequency resolution 375 Hz and time resolution 0.33 ms. For measuring AUDs, we used the following settings: sampling frequency 48 kHz, a Hamming window, Fast Fourier Transform 1024 points, frame 50%, overlap 93.75%, providing frequency resolution 47 Hz and time resolution 1.33 ms. As minimum fundamental frequency of USVs always exceeded 10 kHz, before measurements of USVs all wav-files were subjected to 10 kHz high-pass filtering, to remove low-frequency noise. As minimum fundamental frequency of AUDs always exceeded 0.1 kHz, before measurements of AUDs all wav-files were subjected to 0.1 kHz high-pass filtering, to remove low-frequency noise. All acoustic measurements were exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

For each USV and AUD we measured, in the spectrogram window of Avisoft, the duration with the standard marker cursor, and the maximum fundamental frequency (f_{0max}), the minimum fundamental

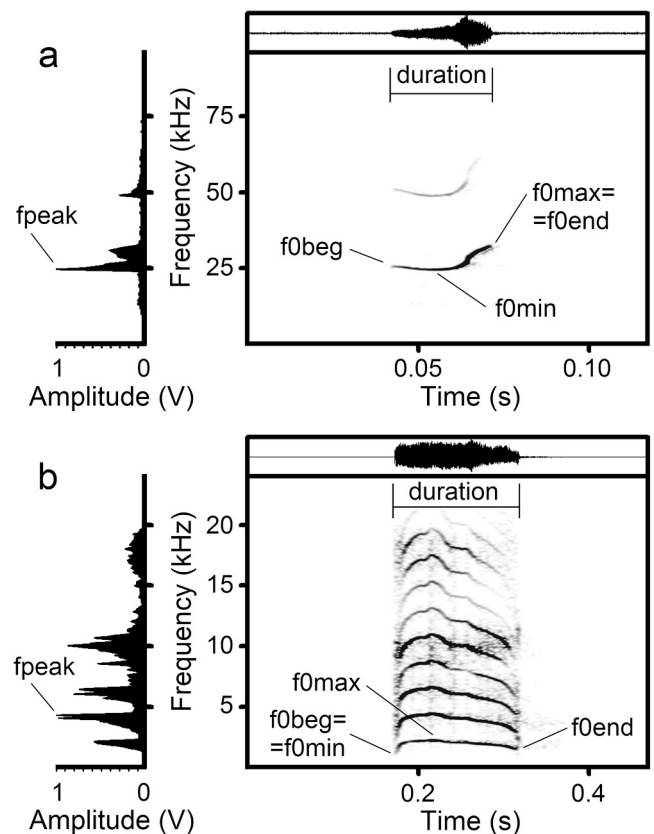


Fig. 1. Measured variables for yellow steppe lemmings: (a) ultrasonic (USV) call; (b) audible (AUD) call. Spectrogram (right) and mean power spectrum of the entire call (left). Designations: duration – call duration; f_{0beg} – the fundamental frequency at the onset of a call; f_{0max} – the maximum fundamental frequency; f_{0end} – the fundamental frequency at the end of a call; f_{0min} – the minimum fundamental frequency; f_{peak} – the frequency of maximum amplitude. Spectrogram was created using sampling frequency 192 kHz (for USV) or 48 kHz (for AUD), Hamming window, Fast Fourier Transform (FFT) 1024 points, frame 50%, overlap 96.87%.

frequency (f_{0min}), the fundamental frequency at the onset of a call (f_{0beg}), and the fundamental frequency at the end of a call (f_{0end}) with the reticule cursor (Fig. 1). For each USV and AUD we measured, in the power spectrum window of Avisoft, the frequency of maximum amplitude (f_{peak}) from the call's mean power spectrum (Fig. 1).

2.6. Statistics

Statistical analyses have been conducted using R Statistical Environment version 4.0.3 (R Core Team, 2020). Data are presented as mean \pm SE. All tests were two-tailed, with a significance level of 0.05. We used a one-way ANOVA to estimate the effect of individuality (= animal identity) on the acoustics of USVs and AUDs for each age class separately ($n = 12$ age classes along ontogeny from neonates to adults). To verify the individuality rates of the calls and to calculate the probability of the assignment of calls to the correct individual, we performed a total of 24 standard discriminant function analyses (DFAs), one DFA per age class per call type, USV or AUD. For each DFA, we estimated the cross-validated error rate using jackknife linear discriminant function analysis (Efron, 1992) implemented in package MASS (Venables and Ripley, 2002) in R (R Core Team, 2020). To validate our DFA results, we calculated the random values of correct assignment of calls to individual callers separately for USVs and AUDs by applying randomization procedures with macros created in R. The random values were averaged from DFAs carried out on 1000 randomized permutations on the data

Table 1

One-way ANOVA results for the effect of caller identity on the acoustic variables of ultrasonic calls of yellow steppe lemmings from 12 age classes. Designations: USVs – ultrasonic calls; 1–12 – the 12 age classes along ontogeny from neonates to adults in days (1–4 d, 5–8 d, 9–12 d, 13–16 d, 17–20 d, 21–24 d, 25–28 d, 29–32 d, 33–36 d, 37–40 d; 41–60 d and adults over 90 d); duration – call duration; f0beg – the fundamental frequency at the onset of a call; f0max – the maximum fundamental frequency; f0end – the fundamental frequency at the end of a call; f0min – the minimum fundamental frequency; fpeak – the frequency of maximum amplitude. The only one non-significant effect is labeled in bold.

| Age class | Acoustic variables of USVs | | | | | |
|-----------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Duration | f0beg | f0max | f0end | f0min | fpeak |
| 1 | $F_{9,90} = 11.94, p < 0.001$ | $F_{9,90} = 11.04, p < 0.001$ | $F_{9,90} = 10.62, p < 0.001$ | $F_{9,90} = 3.07, p = 0.003$ | $F_{9,90} = 4.42, p < 0.001$ | $F_{9,90} = 4.56, p < 0.001$ |
| 2 | $F_{9,90} = 8.22, p < 0.001$ | $F_{9,90} = 8.38, p < 0.001$ | $F_{9,90} = 9.43, p < 0.001$ | $F_{9,90} = 3.48, p < 0.001$ | $F_{9,90} = 10.06, p < 0.001$ | $F_{9,90} = 4.67, p < 0.001$ |
| 3 | $F_{9,90} = 8.03, p < 0.001$ | $F_{9,90} = 5.24, p < 0.001$ | $F_{9,90} = 10.52, p < 0.001$ | $F_{9,90} = 4.92, p < 0.001$ | $F_{9,90} = 4.86, p < 0.001$ | $F_{9,90} = 3.77, p < 0.001$ |
| 4 | $F_{9,90} = 8.76, p < 0.001$ | $F_{9,90} = 2.12, p = 0.035$ | $F_{9,90} = 4.37, p < 0.001$ | $F_{9,90} = 3.98, p < 0.001$ | $F_{9,90} = 4.03, p < 0.001$ | $F_{9,90} = 1.61, p = 0.13$ |
| 5 | $F_{9,90} = 12.23, p < 0.001$ | $F_{9,90} = 9.49, p < 0.001$ | $F_{9,90} = 13.10, p < 0.001$ | $F_{9,90} = 5.93, p < 0.001$ | $F_{9,90} = 12.10, p < 0.001$ | $F_{9,90} = 11.39, p < 0.001$ |
| 6 | $F_{9,90} = 9.29, p < 0.001$ | $F_{9,90} = 9.74, p < 0.001$ | $F_{9,90} = 15.63, p < 0.001$ | $F_{9,90} = 9.61, p < 0.001$ | $F_{9,90} = 11.13, p < 0.001$ | $F_{9,90} = 7.35, p < 0.001$ |
| 7 | $F_{9,90} = 7.85, p < 0.001$ | $F_{9,90} = 5.79, p < 0.001$ | $F_{9,90} = 7.90, p < 0.001$ | $F_{9,90} = 8.02, p < 0.001$ | $F_{9,90} = 6.72, p < 0.001$ | $F_{9,90} = 4.66, p < 0.001$ |
| 8 | $F_{9,90} = 4.78, p < 0.001$ | $F_{9,90} = 6.55, p < 0.001$ | $F_{9,90} = 6.24, p < 0.001$ | $F_{9,90} = 3.03, p = 0.003$ | $F_{9,90} = 5.83, p < 0.001$ | $F_{9,90} = 4.39, p < 0.001$ |
| 9 | $F_{9,90} = 9.17, p < 0.001$ | $F_{9,90} = 6.79, p < 0.001$ | $F_{9,90} = 12.67, p < 0.001$ | $F_{9,90} = 6.49, p < 0.001$ | $F_{9,90} = 7.67, p < 0.001$ | $F_{9,90} = 6.68, p < 0.001$ |
| 10 | $F_{9,90} = 13.33, p < 0.001$ | $F_{9,90} = 9.24, p < 0.001$ | $F_{9,90} = 7.35, p < 0.001$ | $F_{9,90} = 5.29, p < 0.001$ | $F_{9,90} = 10.69, p < 0.001$ | $F_{9,90} = 4.27, p < 0.001$ |
| 11 | $F_{9,90} = 4.44, p < 0.001$ | $F_{9,90} = 6.11, p < 0.001$ | $F_{9,90} = 14.08, p < 0.001$ | $F_{9,90} = 10.71, p < 0.001$ | $F_{9,90} = 8.93, p < 0.001$ | $F_{9,90} = 10.64, p < 0.001$ |
| 12 | $F_{9,90} = 2.43, p = 0.016$ | $F_{9,90} = 3.32, p = 0.002$ | $F_{9,90} = 5.30, p < 0.001$ | $F_{9,90} = 12.80, p < 0.001$ | $F_{9,90} = 3.25, p = 0.002$ | $F_{9,90} = 4.59, p < 0.001$ |

Table 2

One-way ANOVA results for the effect of caller identity on acoustic variables of the audible calls (AUDs) of yellow steppe lemmings from 12 age classes. Designations: AUDs – audible calls; 1–12 – the 12 age classes along ontogeny from neonates to adults in days (1–4 d, 5–8 d, 9–12 d, 13–16 d, 17–20 d, 21–24 d, 25–28 d, 29–32 d, 33–36 d, 37–40 d; 41–60 d and adults over 90 d); duration – call duration; f0beg – the fundamental frequency at the onset of a call; f0max – the maximum fundamental frequency; f0end – the fundamental frequency at the end of a call; f0min – the minimum fundamental frequency; fpeak – the frequency of maximum amplitude.

| Age class | Acoustic variables of AUDs | | | | | |
|-----------|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|
| | Duration | f0beg | f0max | f0end | f0min | fpeak |
| 1 | $F_{9,90} = 3.05, p = 0.003$ | $F_{9,90} = 5.26, p < 0.001$ | $F_{9,90} = 3.21, p = 0.002$ | $F_{9,90} = 2.75, p = 0.007$ | $F_{9,90} = 9.86, p < 0.001$ | $F_{9,90} = 3.34, p = 0.001$ |
| 2 | $F_{9,90} = 4.34, p < 0.001$ | $F_{9,90} = 4.45, p < 0.001$ | $F_{9,90} = 32.91, p < 0.001$ | $F_{9,90} = 6.41, p < 0.001$ | $F_{9,90} = 4.62, p < 0.001$ | $F_{9,90} = 24.29, p < 0.001$ |
| 3 | $F_{9,90} = 3.16, p = 0.002$ | $F_{9,90} = 15.78, p < 0.001$ | $F_{9,90} = 3.50, p < 0.001$ | $F_{9,90} = 4.46, p < 0.001$ | $F_{9,90} = 15.87, p < 0.001$ | $F_{9,90} = 8.31, p < 0.001$ |
| 4 | $F_{9,90} = 3.25, p = 0.002$ | $F_{9,90} = 7.11, p < 0.001$ | $F_{9,90} = 8.83, p < 0.001$ | $F_{9,90} = 4.76, p < 0.001$ | $F_{9,90} = 9.28, p < 0.001$ | $F_{9,90} = 4.43, p < 0.001$ |
| 5 | $F_{9,90} = 3.32, p = 0.002$ | $F_{9,90} = 12.56, p < 0.001$ | $F_{9,90} = 3.52, p < 0.001$ | $F_{9,90} = 2.59, p = 0.011$ | $F_{9,90} = 15.83, p < 0.001$ | $F_{9,90} = 2.51, p = 0.013$ |
| 6 | $F_{9,90} = 8.57, p < 0.001$ | $F_{9,90} = 9.09, p < 0.001$ | $F_{9,90} = 6.21, p < 0.001$ | $F_{9,90} = 3.30, p = 0.002$ | $F_{9,90} = 8.07, p < 0.001$ | $F_{9,90} = 10.25, p < 0.001$ |
| 7 | $F_{9,90} = 2.48, p = 0.014$ | $F_{9,90} = 8.19, p < 0.001$ | $F_{9,90} = 6.67, p < 0.001$ | $F_{9,90} = 4.92, p < 0.001$ | $F_{9,90} = 7.99, p < 0.001$ | $F_{9,90} = 6.14, p < 0.001$ |
| 8 | $F_{9,90} = 3.39, p = 0.001$ | $F_{9,90} = 6.15, p < 0.001$ | $F_{9,90} = 9.17, p < 0.001$ | $F_{9,90} = 3.75, p < 0.001$ | $F_{9,90} = 11.62, p < 0.001$ | $F_{9,90} = 6.80, p < 0.001$ |
| 9 | $F_{9,90} = 6.16, p < 0.001$ | $F_{9,90} = 3.57, p < 0.001$ | $F_{9,90} = 5.10, p < 0.001$ | $F_{9,90} = 4.08, p < 0.001$ | $F_{9,90} = 4.66, p < 0.001$ | $F_{9,90} = 10.40, p < 0.001$ |
| 10 | $F_{9,90} = 7.01, p < 0.001$ | $F_{9,90} = 2.10, p = 0.037$ | $F_{9,90} = 6.74, p < 0.001$ | $F_{9,90} = 4.28, p < 0.001$ | $F_{9,90} = 3.28, p = 0.002$ | $F_{9,90} = 6.90, p < 0.001$ |
| 11 | $F_{9,90} = 4.12, p < 0.001$ | $F_{9,90} = 7.50, p < 0.001$ | $F_{9,90} = 4.95, p < 0.001$ | $F_{9,90} = 5.26, p < 0.001$ | $F_{9,90} = 7.02, p < 0.001$ | $F_{9,90} = 11.73, p < 0.001$ |
| 12 | $F_{9,90} = 2.10, p = 0.038$ | $F_{9,90} = 5.86, p < 0.001$ | $F_{9,90} = 11.60, p < 0.001$ | $F_{9,90} = 2.82, p = 0.006$ | $F_{9,90} = 7.54, p < 0.001$ | $F_{9,90} = 2.74, p = 0.007$ |

sets as described by Solow (1990).

To assess the effect of age on individuality of USVs and AUDs, we ranked the age classes in ascending order from 1 to 12 and analyzed the effects of this ranked variable (factor Age) and the type of call, USV vs AUD (factor Call type) on the rates of classifying the calls to correct callers. We evaluated two separate models with the same predictors (factors Age and Call Type with their interactions) and different responses: the correct classifying rate from standard DFA in the first model and the correct classifying rate from jackknife DFA in the second model. Since the dependent variables didn't fit normal or Poisson distributions and the correct classifying rates for different individuals were non-independent, we used linear permutation models in the LmPerm package (Wheeler and Torchiano, 2016). Finally, we estimated the linear relationships between factor Age and the correct classifying rates of USVs and AUDs to individual callers from the standard and jackknife DFAs using linear permutation models. We calculated Spearman rank order correlation coefficients to estimate the effect sizes of the linear correlations.

3. Results

Based on ANOVA results, all USV and AUD acoustic variables were strongly affected by caller identity at any age class for the exclusion of fpeak of USVs at age class 4 (Tables 1 and 2). These ANOVA results indicate that, at each age class, the acoustic variables of USVs and AUDs provided potential for discriminating individuals by voice. So, we

Table 3

DFA-based percent (Mean ± SE) of correct classifying to individual for ultrasonic and audible calls of yellow steppe lemmings from 12 age classes, results of jackknife DFA (for validation of DFA-based classifying of calls to individual) and results of linear permutation models (for the effects of factors age and call type, ultrasonic vs audible, on the classifying success of calls to individual callers, n = 10 callers per age class). Designations: USVs – ultrasonic calls; AUDs – audible calls; 1–12 – the 12 age classes along ontogeny from neonates to adults in days (1–4 d, 5–8 d, 9–12 d, 13–16 d, 17–20 d, 21–24 d, 25–28 d, 29–32 d, 33–36 d, 37–40 d; 41–60 d and adults over 90 d).

| Age class | Standard DFA | | Jackknife DFA | |
|---------------|---------------------------|----------|---------------------------|----------|
| | USV | AUD | USV | AUD |
| 1 | 57 ± 7.0 | 41 ± 7.7 | 50 ± 9.1 | 28 ± 9.2 |
| 2 | 53 ± 7.7 | 58 ± 5.5 | 40 ± 7.7 | 45 ± 6.4 |
| 3 | 47 ± 8.6 | 53 ± 7.5 | 32 ± 8.8 | 31 ± 6.0 |
| 4 | 55 ± 4.5 | 45 ± 9.0 | 47 ± 5.8 | 36 ± 8.1 |
| 5 | 57 ± 7.9 | 49 ± 7.1 | 51 ± 9.8 | 30 ± 7.9 |
| 6 | 52 ± 5.3 | 57 ± 6.3 | 37 ± 7.2 | 38 ± 6.1 |
| 7 | 53 ± 8.2 | 47 ± 7.8 | 46 ± 8.5 | 28 ± 7.3 |
| 8 | 42 ± 8.0 | 56 ± 6.9 | 31 ± 8.7 | 44 ± 7.9 |
| 9 | 59 ± 4.3 | 51 ± 8.5 | 40 ± 5.8 | 42 ± 7.9 |
| 10 | 62 ± 6.8 | 56 ± 7.6 | 45 ± 7.5 | 38 ± 9.3 |
| 11 | 52 ± 8.0 | 50 ± 6.0 | 33 ± 8.7 | 32 ± 7.6 |
| 12 | 42 ± 8.3 | 48 ± 5.1 | 29 ± 7.8 | 30 ± 6.0 |
| Age | Estimate = -0.05, p = 0.8 | | Estimate = -0.48, p = 0.1 | |
| Call type | Estimate = -0.08, p = 0.9 | | Estimate = -2.46, p = 0.1 | |
| Age*Call type | Estimate = 0.3, p = 1.0 | | Estimate = 0.5, p = 0.07 | |

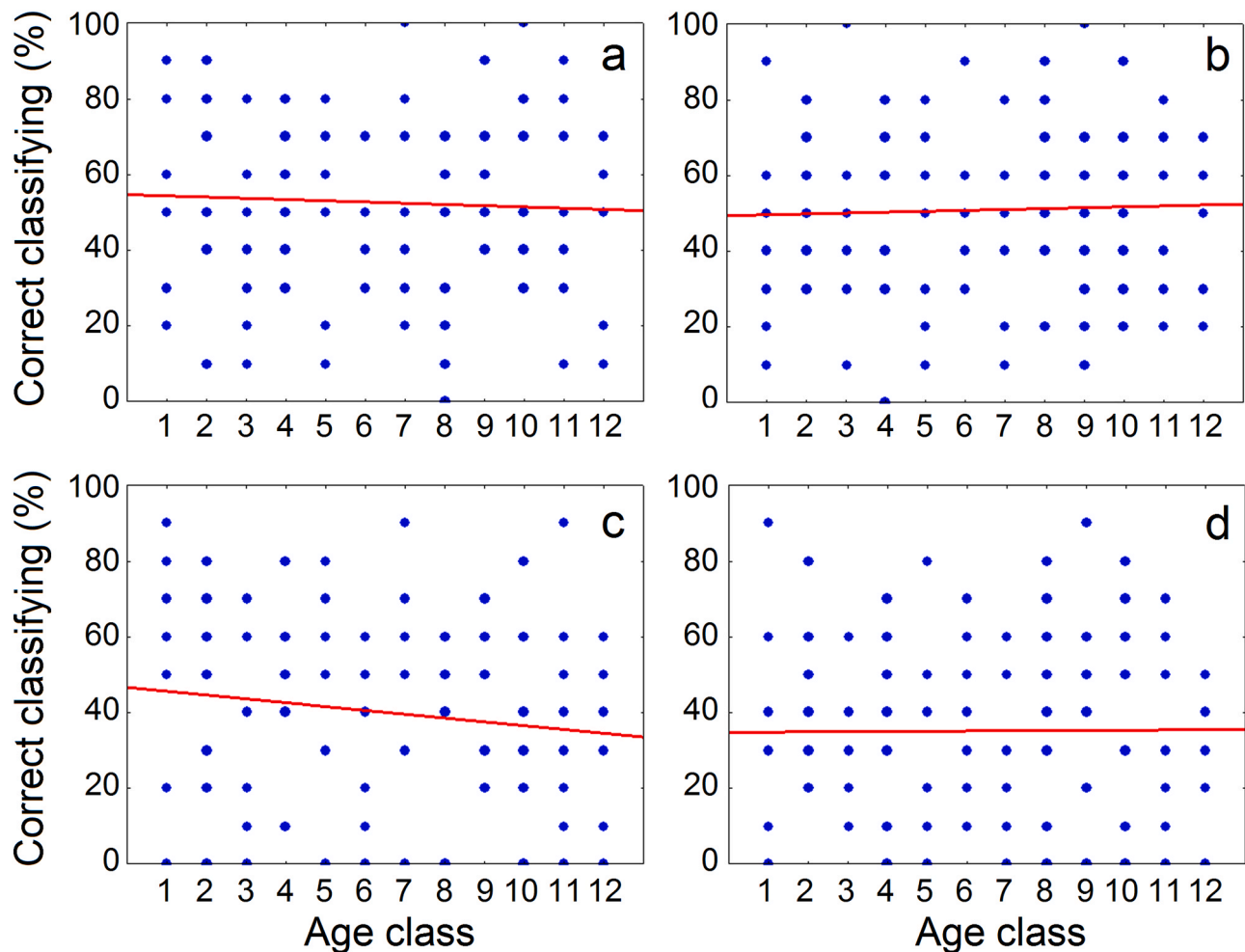


Fig. 2. Scatterplots illustrating the relationships between factor Age and percent of classifying of calls to correct caller (a) for USV and standard DFA; (b) for AUD and standard DFA; (c) for USV and jackknife DFA; (d) for AUD and jackknife DFA. Linear regression lines are shown. All relationships are non-significant ($p > 0.1$).

included all the six measured acoustic variables of USVs and AUDs in both standard and jackknife DFAs for acoustic individuality.

Among age classes, the mean percent of correct classifying of calls to individual with standard DFA varied from 62% to 42% for USVs and from 58% to 41% for AUDs (Table 3). The random values of correct classifying ranged among age classes from 27.24% to 28.35% for USVs and from 27.85% to 28.64% for AUDs (permutation test, 1000 permutations). However, in many individuals, 16 (13.3%) of USV callers and 17 (14.2%) of AUD callers, the classifying success of calls to correct caller was below chance level, so, their calls could not be discriminated from the samples of USVs or AUDs. After applying jackknife DFA cross-validation, the mean percent of correct classifying of calls to individual decreased to 51–29% for USVs and to 45–28% for AUDs (Table 3).

Permutation test showed that the degree of acoustic individuality of USVs and AUDs revealed based on the standard and jackknife DFAs, did not change across age classes (Table 3). Permutation test also showed the lack of differences in the degree of acoustic individuality in USVs vs AUDs at any age class (Table 3).

Relationships between Age and the correct assignment rates from both standard and jackknife DFAs were non-significant for USVs (Estimate = -0.3 , $r_s = -0.04$, $p = 0.7$, Estimate = -1.0 , $r_s = -0.14$, $p = 0.1$, respectively) as well as for AUDs (Estimate = 0.2 , $r_s = 0.03$, $p = 0.8$, Estimate = 0.1 , $r_s = 0.01$, $p = 1.0$, respectively, all p values were calculated using permutation tests) (Fig. 2).

4. Discussion

We found that, against expectations, isolation-induced ultrasonic (USVs) and audible (AUDs) calls of yellow steppe lemmings did not show the predicted trend towards increasing individuality from pups to adults. Instead, acoustic individuality of both USVs and AUDs remained uniformly low across ontogeny. Degrees of individualization of both USVs and AUDs among all age classes were higher than values of classifying by chance. At the same time, many particular callers could not be discriminated from the total samples of USVs or AUDs by their calls.

These results are surprising and unusual for either AUDs or USVs of rodents. Many studies report the individualized audible calls in rodents, as e.g., in ground squirrels (Hare, 1998; Matrosova et al., 2009, 2010; Schneiderová et al., 2017), marmots (Nikol'skii and Suchanova, 1994; Blumstein and Munos, 2005; Matrosova et al., 2011), Guinea pigs (Tokumaru et al., 2004), capybaras (Lacerda et al., 2014), birch mice (Volodin et al., 2019a) and dormice (Ancillotto and Russo, 2016). Even very simple in the acoustic structure alarm calls of speckled ground squirrels *Spermophilus suslicus* could be classified to correct callers with 90% accuracy among 20 individuals (Matrosova et al., 2009). However, consistently to our results displaying a low individuality along ontogeny in yellow steppe lemmings, limited data on the audible contact calls of Guinea pigs suggest a low individuality along ontogeny in this rodent (Baklová, 2016). Regarding the individuality of USVs in rodents, data are scarce, however, two studies reported the substantially individualized courtship songs in male domestic mice (Hoffmann et al., 2012;

Marconi et al., 2020).

Aside rodents, the ontogeny of acoustic individuality was studied in ruminants, primarily in domestic goats *Capra hircus* (Briefer and McElligott, 2011) and goitred gazelles *Gazella subgutturosa* (Lapshina et al., 2012). Similar to rodents, ruminants do not experience production vocal learning (Janik and Slater, 1997, 2000), so data on ontogeny of acoustic individuality in ruminants are better comparable with respective data on rodents than e.g., respective data on vocal learners, such as whales (Tyack, 1997), seals (Charrier et al., 2003) or bats (Knörnschild et al., 2012). Overall, data on ruminants suggest that acoustic individuality of the contact calls is higher where the social environment of animal is more complex and where the risk of loss of mother-offspring contact is higher (Sibiriyakova et al., 2017). In ruminant hider species, as domestic goats or goitred gazelles, acoustic individuality of the contact and distress calls is moderately high along ontogeny (Briefer and McElligott, 2011; Lapshina et al., 2012; Volodin et al., 2017b). In a super-follower species, the saiga *Saiga tatarica*, individuality of the contact calls is extremely high (with classifying success up to 99% for 18 mothers and 94% for 18 neonates, Sibiriyakova et al., 2017). For saiga, the stated at birth individual vocal signature is critically important, because in a day after birth, the newborn follows a mother in a herd of a few hundred individuals, where a loss of contact with a mother means a great risk of mortality for the young and reproduction failure for the female.

Yellow steppe lemmings are social animals living in family-based colonies founded by a parental pair with descendants of a few subsequent litters (Smorkatcheva et al., 2008, 2009). The USVs and AUDs used for communication with family members at weak disturbance in home cages (e.g., re-placement of a hide to another corner), are of the same acoustic structure as those recorded during experiments in this study (IAV and EAV, unpubl. data). So, the individualistic acoustic traits are reasonably expected to be present in the calls used for personalized acoustic communication between family members. However, our results suggest that both USVs and AUDs of yellow steppe lemmings were non-individualized. Probably, yellow steppe lemmings possess group vocal signatures rather than individual vocal signatures, as in bats (Knörnschild et al., 2012) and in ruminants (Volodin et al., 2014). Otherwise, yellow steppe lemmings may use, instead of vocalizations, another communicative channel for recognizing group members, e.g., a common odor. The discovered low individuality of USVs and AUDs in a social rodent species is unusual and needs a further investigation of a broader numbers of species of rodents.

Previous studies revealed a high potential of yellow steppe lemming as a wild-type animal model for biomedical research. Both pup and adult yellow steppe lemmings produce USVs and AUDs of comparable acoustic structure between ages (Yurlova et al., 2020; Klenova et al., 2021a; Volodin et al., 2021b). In both pups and adults, emission of USVs can be induced in the same easily modeled standard situation of isolation of the focal animal on an unfamiliar territory for few minutes. This allows conducting experiments with recording and analysis of USVs at all age classes of yellow steppe lemmings by using the uniform experimental protocol, what makes the results of these tests well-comparable (Yurlova et al., 2020; Klenova et al., 2021a; Volodin et al., 2021b). This is distinctive from the most widespread rat and mice animal models, in which pups and adults produce USVs in different test situations (Wöhr, 2014; Weiner et al., 2016). We revealed that factor individuality has a minimal effect on the acoustics of USVs in yellow steppe lemming at all ages from neonates to adults, what enhances potential value of this animal model (without the confounding effects of individuality on USVs) for biomedical research.

CRedit authorship contribution statement

Ilya A. Volodin: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Daria D. Yurlova:** Formal analysis, Investigation, Writing – original draft,

Writing – review & editing. **Olga G. Ilchenko:** Investigation, Resources, Writing – original draft, Writing – review & editing. **Nina A. Vasilieva:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Elena V. Volodina:** Conceptualization, Validation, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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