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Original investigation

Genetic and alarm call diversity across scattered populations of speckled ground squirrels (*Spermophilus suslicus*)

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ABSTRACT

Alarm calls of ground squirrels are innate signals, showing substantial geographical variation across populations without the masking effects of sex and age- related variation. This makes them a convenient model for studying population genetic effects on the evolution of alarm communication. We compared data on the alarm call structure and the mitochondrial DNA (mtDNA) complete control region (C-region) (998–1002 bp) polymorphism in the same 90 individual speckled ground squirrels (*Spermophilus suslicus*) across 6 populations (15 individuals per population), separated by distances from 12 to 1274 km. We calculated acoustic distances between each pair of populations based on acoustic variables of alarm calls, averaged for each individual using Euclidean distances of population centroids, in the space of canonical axes of discriminant function analysis. Genetic distances ranged of 0–1.1% within populations and of 0.5–4.9% between populations. Prominent differences were found between eastern and western populations separated by the Dnieper River. Both genetic and acoustic distances showed a significant positive correlation with geographical among populations. Positive correlation between acoustic and genetic distances did not reach significance. These results support effects of ecological selection on the alarm call variables rather than the genetic drift hypothesis. In addition, these results support the current taxonomic separation between subspecies of speckled ground squirrels differing in diploid chromosome sets $2n = 34$ (*Spermophilus suslicus guttatus*) and $2n = 36$ (*Spermophilus suslicus odessanus*).

Introduction

Relations between geographical variation in vocal and genetic data attract a wide research interest focused on the evolution of acoustic communication in rodents (mole-rats: Nevo et al., 1987; singing mice: Campbell et al., 2010; grounds squirrels: Eiler and Banack, 2004; Schneiderová and Policht, 2012a,b; domestic mice: von Merten et al., 2014). In general, acoustic divergence may be predicted by different selection regimes: ecological selection, sexual selection, a combination of both and by neutral evolution, i.e., genetic drift (Wilkins et al., 2013). Particular selection regimes do not specifically relate to particular call functions, e.g., acoustic divergence of male courtship calls are not necessarily related to sexual selection and may be explained by genetic drift (Campbell et al., 2010).

Alarm calls of ground squirrels are strongly subject to geographical variations within and between species, which makes them excellent vocal indicators of species (Matrosova et al., 2012b; Schneiderová and Policht, 2012a,b), subspecies (Eiler and Banack, 2004; Nikol'skii et al., 2007), and even populations (Eiler and Banack, 2004; Nikol'skii et al., 2007; Matrosova et al., 2012a). These differences are expected to be genetically predetermined, as interspecies hybridization in ground squirrels results in intermediate acoustic characteristics between parental patterns of alarm calls (Koeppl et al., 1978; Nikol'skii et al., 1984; Titov et al., 2005). In addition, the vocal learning in ground squirrels could not be proved even by direct cross-foster interspecies parenting experiments (Matocha, 1975). The speckled ground squirrel has been

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Table 1
Characteristics of 6 study populations.

Population	Location	Habitat	Year
Zaraysk (ZAR)	54°47′68″N 38°42′23″E	An open dry floodable meadow near the village Velikoe pole, Zaraysk district, Moscow region, Russia	2005–2009
Lipetsk (LIP)	52°36′28″N 39°26′38″E	A municipal cemetery established in 1980 in place of farmlands near the village Kosyrevka, Lipetsk region, Russia	2010
Michurinsk (MIC)	52°51′44″N 40°47′01″E	Remains of a formerly successful colony along the M-6 federal highway near the village Dmitrievka, Michurinsk district, Tambov region, Russia	2013
Novosel'skoe (NOV)	45°20′44″N 28°36′33″E	A pasture with moderate grazing pressure at the low bank of Yalpug Lake near the village Novosel'skoe, Reni district, Odessa region, Ukraine	2013
Ozernoe (OZE)	45°25′92″N 28°40′04″E	A pasture with overgrazed grass stand at the high bank of the Yalpug Lake near the village Ozernoe, Izmail district, Odessa region, Ukraine	2013
Odessa (SOV)	46°20′66″N 30°40′31″E	A waste ground along tram ways in Sovinyon suburb of the city Odessa, Odessa region, Ukraine	2014

previously studied separately for genetic variation among five populations (Matrosova et al., 2014) and for acoustic variation in two populations (Matrosova et al., 2012a). However, the relationships between acoustic and genetic characteristics have not been studied to date in any ground squirrel.

The speckled ground squirrel *Spermophilus suslicus* (Güldenstädt, 1770) is a small-sized (body mass 180–220 g, body length without tail 190–220 mm), diurnal, herbivorous, obligate hibernating sciurid (Lobkov, 1999), which was formerly continuously distributed over the steppes and forest-steppes of Russia, Ukraine, Moldova, Belarus and locally in Poland (Wilson and Reeder, 2005; Helgen et al., 2009). However, the current distribution area is highly fragmented into separate colonies due to human activities (Nedosekin, 2007; Shekarova et al., 2008; Matrosova et al., 2012a). Three to five subspecies have been previously recognized in this species (Wilson and Reeder, 2005; Helgen et al., 2009). The species range is subdivided by the insurmountable geographical barrier of the Dnieper River into the eastern and western parts. Eastern speckled ground squirrels *Spermophilus suslicus guttatus* (Pallas, 1770) have a diploid chromosome set $2n = 34$, $NF = 68$, whereas the western speckled ground squirrels *Spermophilus suslicus odessanus* (Nordmann, 1840) have $2n = 36$, $NF = 72$ (Denisov et al., 1969; Lyapunova and Vorontsov, 1970). Recent mtDNA barcoding study confirmed the noticeable level of genetic differences between the eastern and western ground squirrels (Ermakov et al., 2015).

Alarm calls of speckled ground squirrels consist of weakly modulated tonal notes about 200 ms in duration, with fundamental frequencies ranging from 8 to 10 kHz (Fig. 1), that are typically produced in series with intervals substantially longer than the duration of the notes themselves (Nikol'skii, 1979; Nikol'skii et al., 1984; Volodin, 2005). Very rarely can the alarm calls be complicated with an ultrasonic component (Matrosova et al., 2012b). All previous studies of the acoustic variation of alarm calls in speckled ground squirrels have been conducted on colonies from the eastern part of the distribution area (Nikol'skii, 1979; Nikol'skii et al., 1984; Volodin, 2005; Matrosova et al., 2007, 2009, 2011, 2012a,b). At the same time, the acoustic variation of alarm calls in the western ground squirrels has not been described to date.

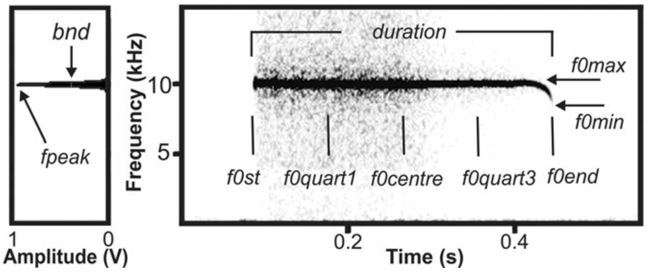

Fig. 1. Measured variables of the alarm call. Left: the mean power spectrum; right: spectrogram. *f0max* – the maximum fundamental frequency; *f0min* – the minimum fundamental frequency; *f0st* – the fundamental frequency at the beginning; *f0quart1* – the fundamental frequency at the 1st quarter; *f0centre* – the fundamental frequency in the middle; *f0quart3* – the fundamental frequency at the 3rd quarter; *f0end* – the fundamental frequency at the end; *fpeak* – the maximum amplitude frequency; *bnd* – the bandwidth of maximum amplitude frequency.

In the lack of differences across sex and age-classes in the alarm call structure in the eastern speckled ground squirrels (Matrosova et al., 2011, 2012a), the geographic-related variation affects all the age- and sex-classes uniformly. Significant 15% differences (about 1 kHz) in the alarm call fundamental frequency were found between two eastern populations of speckled ground squirrels (Matrosova et al., 2012a). These differences were observed in both pups and adults and in both sexes. Similarly noticeable genetic differences (5 nucleotide substitutions) in the full-size control region of mitochondrial DNA (C-region) were found between these populations, although within populations, the C-region was conservative (Matrosova et al., 2014). Comparative studies of nucleotide