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Karyotype, chromosome structure, reproductive modalities of three Southern Eurasian populations of the common lacertid lizard, *Zootoca vivipara* (Jacquin, 1787)

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Abstract. According to a hypothesis of the evolution of viviparity the lacertid lizard *Zootoca vivipara*, rare relict oviparous populations of the species might occur in southern-eastern part of its distribution area. Such a hypothesis has been verified by comparing the karyotype, chromosome structure, and reproductive modality of three populations of south-eastern part of Russia, including Altai and neighbouring regions, where small territories remained isolated during the Pleistocene cooling and where Pleistocenic fossils of *Z. vivipara* have been found. The chromosomal study was carried out by conventional staining method and banding methods, namely C-banding and sequential staining of C-banding+ fluorochromes, CMA_3 and DAPI. All studied females displayed viviparous reproductive modality and showed a karyotype of 2N = 35 acrocentric chromosomes, with a Z_1Z_2W sex chromosome system. Chromosome W was subtelocentric. No inter-population variability on karyotype and heterochromatin distribution and composition was observed. From the obtained data the three studied south-eastern Russian viviparous populations belong to the Russian viviparous form of *Z. v. vivipara*.

Keywords. *Zootoca vivipara,* karyotype, heterochromatin, viviparous modality, distribution.

INTRODUCTION

The wide-ranged Eurasian lacertid species *Zootoca vivipara* (Jaquin, 1787) present oviparous and viviparous populations, either showing a high geographical variability in karyotype's features (Chevalier et al., 1979; Kupriyanova, 1986, 1990; Odierna et al., 1993). Along its vast range rather morphologically similar individuals from different populations of *Z.*

vivipara can be recognized on the basis of combined analysis of their karyotypic features and reproductive mode. In western and central Europe all so far discovered oviparous and viviparous chromosomal forms appear to have distinct distribution range (parapatric, allopatric and mosaic distributions), some of them inhabiting small areas, others resulting rare within a country and needing protection there (Odierna et al., 1993, 1998; Kupriyanova et al., 2005a, 2006) (see Table 1). Recent modern advanced cytogenetical investigations also have given information. It has been shown that oviparous and viviparous populations of different forms are characterized by several chromosome markers (AT and GC rich clusters of DNA) allowing to (1) identify with precision these forms and subspecies; (2) clarify their distribution; (3) elucidate a possible role of morphological and molecular chromosome change in the processes of subspeciation and form-formation and in evolution of viviparity (Odierna et al., 2001, 2004; Kupriyanova, 2004; Kupriyanova et al., 2005a).

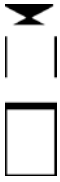
Concerning *Z. vivipara* populations inhabiting Russia, combined data on their reproductive modality, karyotype and chromatinic markers have been obtained mainly in specimens from western and north-western parts of Russia: two different chromosomal forms of *Z. v. vivipara* with viviparous mode of reproduction were found (Kupriyanova et al. 1995, Kupriyanova, 2004; Kupriyanova et al., 2007). Present paper shows the results of a karyological and reproductive analysis on three previously unstudied *Z. vivipara* populations from southern-central part of European Russia, Altai and neighbouring regions. Data on populations from these areas are of particular interest as may help: to find some additional characters of different forms and subspecies; to clarify the structure and biogeography of the species; to identify the centre(s) of their origin(s) and refugium(a); to give insight on the role of chromosomal changes in the evolution of sex chromosomes and viviparity, in form(s)-formation. Furthermore, according to a hypothesis of the evolution of viviparity in this lacertid lizard (Heulin et al., 1993), rare relict oviparous populations of the species might occur in southern-eastern part of its distribution areas. In this regard, Altai and neighbouring regions are very interesting as they include small territories, with mountain-taiga landscape, which remained isolated during Pleistocene cooling (Sinizin, 1962), and where Pleistocenic fossils of *Z. vivipara* have been found (Putieva and Chkhikvadze, 1990).

MATERIALS AND METHODS

The karyotypes analysed come from five females and one male of *Z. vivipara* from Tambov region, 50 km north of Tambov, 53°N, 41°E (southern-central part of European Russia) collected in May 2004 (Population 1 in Fig. 1); from five females and two males of *Z. vivipara* from Tuva Republic near Todga lake, 200 km north-east of Kizil, 52°N, 90°E, 2100 m above sea level (southern Siberia, Asian Russia) collected in June 2004 (Population 2 in Fig. 1); from three females and two males of *Z. vivipara* from the border between Tuva and Altai regions, 15 km north-west of Kara-Khol lake, 50°N, 90°E, 2300 m above sea level (southern Siberia, Asian Russia) collected in June 2004 (Population 3 in Fig. 1).

The chromosomes were obtained according to the scraping and air-drying method from intestine, blood and lung tissues (Odierna et al., 1993). The specimens were injected with 0.1% phytohemagglutinin M (Difco) three times during three weeks (0.08 ml/5 g body weight) and then with 0.05% colchicines (0.1 ml/5 g body weight) 1 hour before sacrificing animal. The slides were stained

Table 1. Distribution, taxonomy, reproductive mode and chromosome characters of females from different populations of *Zootoca vivipara* so far known. (Ref. 1 = Chevalier et al., 1979; 2 = Kupriyanova, 1990; 3 = Odierna et al., 1999; 4 = Odierna et al., 2001; 5 = Odierna et al., 2004).

Populations	Subspecies	Reproductive mode	Female chrom. number	Female sex chromosome system	W chromosome morphology and heterochromatin distribution
Western and Central Europe, Scandinavia: Sweden Slovakia	Western form of *Z. v. vivipara* and *Z. v. pannonica*	viviparous	35	Z_1Z_2W (Ref. 1, 3)	
Eastern Europe: Russia, Estonia, Belarus, Ukraine, Hungary, Fennoscandia: Finland, Sweden. Asia	Russian form of *Z. v. vivipara* and *Z. v. sachalinensis*	viviparous	35	Z_1Z_2W (Ref. 2, 3)	
Central Europe: Austria (the type locality), Austrian Pannonian lowland	Austian form of *Z. v. vivipara*	viviparous	35	Z_1Z_2W (Ref. 2)	
Western Europe: Western Pyrenees, Aquitania	Pyreneean form 1 of *Z. v. vivipara*	oviparous	35	Z_1Z_2W (Ref. 3)	
Eastern Pyrenees	Pyreneean form 2 of *Z. v. vivipara*	oviparous	35	Z_1Z_2W (Ref. 3)	
Southern-central Europe: Slovenia, Northeast Italy, Southern Austria	*Z. v. carniolica*	oviparous	36	ZW (Ref. 4)	
Central Europe: Central Hungary (Poland (Mand), Eastern Austria	Hungarian form of *Z. v. vivipara*	viviparous	36	ZW (Ref. 5)	

for 10 min. with a 5% Giemsa solution in pH 7 phosphate buffer. Sumner's indications (1972) were followed for C-banding staining. Sequential staining of C-banding and/or Alu 1 endonuclease digestion + CMA_3+DAPI were conducted according to Odierna et al. (1999). To observe the mode of reproduction three pregnant females from each of studied populations were kept in a terrarium during June and July 2004 up to hatching. The females were reared separately in a plastic terrarium of 30x20x20 cm, equipped with a shelter, dishes of food and water, heated for 6 h/day with a 40W bulb lamp. Terraria were checked for clutches four times a day.

RESULTS AND DISCUSSION

The observations of studied pregnant females of *Z. vivipara* studied in the terrarium and in nature have shown that they were viviparous. Independently of provenance males showed a karyotype of 2N = 36 uniarmed acrocentric (A) chromosomes (Foundamental Number, FN = 36), whereas females had a chromosome set of 2N = 35 acrocentric (A) elements, then possessing a Z_1Z_2W sex chromosome system, with W shaped as an uniarmed acrocentric/subtelocentric (A/ST) macrochromosome. Most of autosomes and W chromosome possessed conspicuous centromeric and tiny telomeric C-bands. Additionally, W constantly had a remarkable interstitial C-band , which was Alu 1 resistant (Fig. 1). After sequential staining of C-banding + DAPI + CMA_3 centromeric C-bands of 14-16 autosomes and W chromosome, as well as the interstitial W heterochromatin, were DAPI positive, then AT rich (Fig. 2), while telomeric C-bands of several autosomes were CMA_3 positive, then GC rich. However, two of telomeric CMA_3 positive loci were more intensively stained than others and corresponding to the regions where NORs were identi-

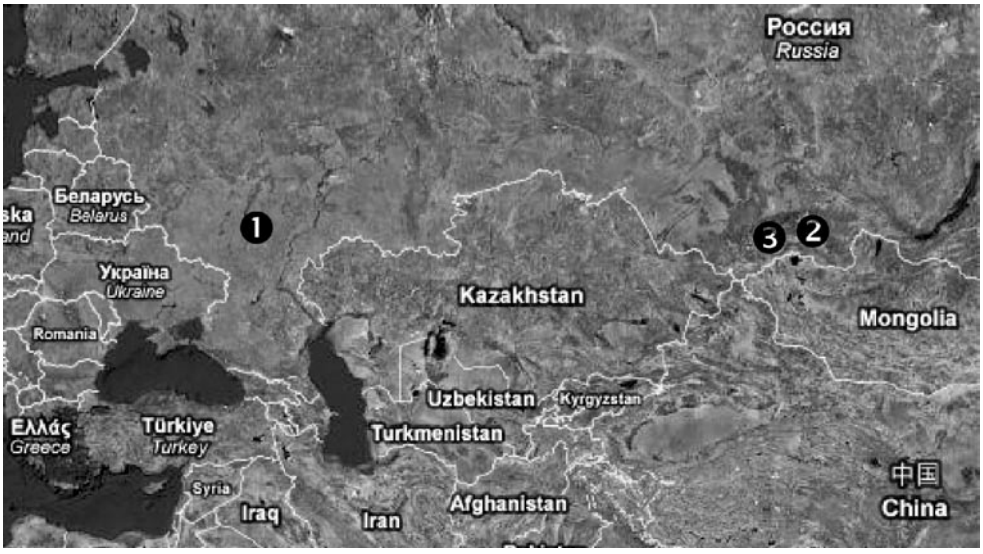


Fig. 1. Distribution of the three studied population of *Z. v. vivipara*: **1**, from Tambov region; **2**, from Tuva Republic near Todga lake; **3**, from Tuva-Altai border, close to Kara-Khol lake.

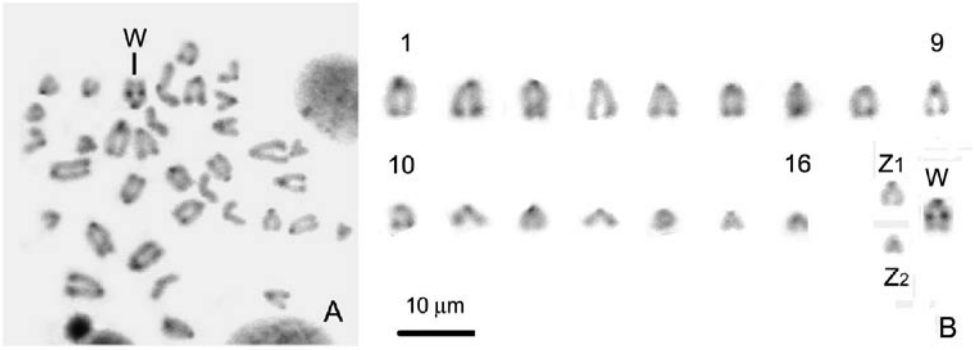


Fig 2. C-Banded Metaphase plate (A) and relative haploid karyotype of a *Z. v. vivipara* female from Altai-Tuva regions.

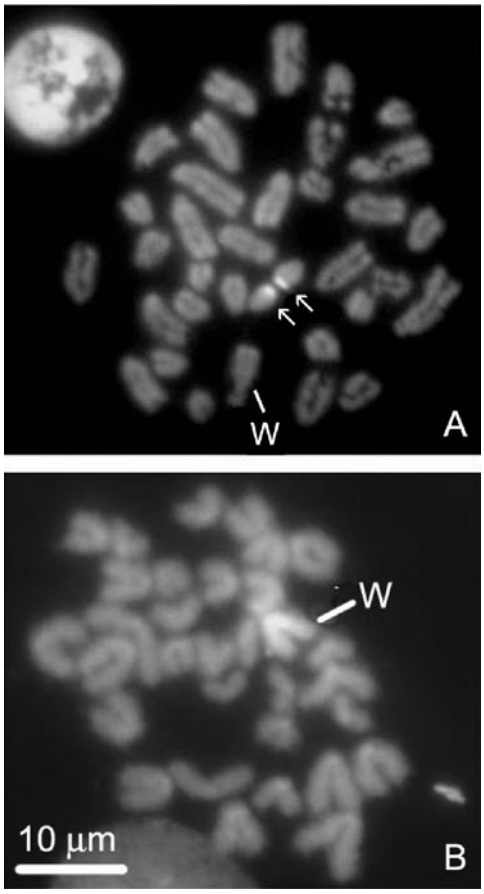


Fig. 3. C-banding +CMA_3 (**A**) and C-banding + DAPI (B) metaphase plate of a *Z. v. vivipara* female from Altai-Tuva regions sequentially stained. The arrows point to chromosomes bearing NORs.

fied (Kupryianova, 1990; Odierna et al., 1998, 2001) (Fig. 2). No variation was observed in these chromatinic markers within and among the three studied populations.

For a comparison detailed data on karyotype and chromatinic markers are known only for populations of *Z. vivipara* inhabiting other regions (see Table 1). The comparison shows that specimens here studied showed similarities in their karyotype and chromatinic markers with those of *Z. vivipara* from north-western part of Russia. The latter also had viviparous modes of reproduction, allowing to assign the three populations here studied to the viviparous Russian form of *Z. v. vivipara* (2N = 35, Z_1Z_2W, and W shaped as A/ST) (Kupriyanova et al., 2005a). This form was for the first time discovered in two populations in Asian and European Russia based only on the rough chromosome morphology (Kupriyanova, 1986). Later, it was also found by chromosome banding methods in one locality in the Transcarpathian region of Ukraine (Kupriyanova, 1990), in one locality in western Estonia (Kupriyanova, 1997), in one locality in eastern Hungary (Puky et al., 2004), in two localities of eastern Finland (Kupriyanova et al., 2005b), in one locality in northern Sweden (Odierna, unpublished evidence) and in two localities in western Russia (Kupriyanova et al., 2007; Kupriyanova and Melashchenko, 2008). Thus this viviparous Russian form of *Z. v. vivipara* has huge distribution area unlike to other discovered viviparous and oviparous forms and subspecies which often have narrow and mosaic distribution ranges.

The present combined reproductive and karyological analysis failed to detect relict oviparous populations of *Z. vivipara* in south-eastern Russian region. However, further combined reproductive and karyological analyses in specimens of *Z. vivipara* from other areas of Altai and neighbouring regions still are needed.

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