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THE COMMON EURASIAN LIZARD *Zootoca vivipara* (JACQUIN, 1787) FROM RUSSIA: SEX CHROMOSOMES, SUBSPECIATION, AND COLONIZATION

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Data on the karyotypes and sex chromosomes of the common Eurasian lizard, *Zootoca vivipara* (Jacquin, 1787) (family Lacertidae), the Kaliningrad Region (Königsberger Gebiet) in the Baltic Sea Basin of Russia are presented. Twenty five viviparous specimens of *Zootoca vivipara* from five geographically separate localities have been identified on the basis of their karyotypes, and as a result two different chromosomal forms of nominative subspecies *Z. v. vivipara* have been recognized: 1. viviparous western form — in female $2n = 35$ with the W-sex submetacentric macrochromosome (SV) (Z_1Z_2W type); 2. viviparous Russian form — in female $2n = 35$, with the W-sex acrocentric/subtelocentric macrochromosome (A/ST) (Z_1Z_2W type).

For the present these are marginal populations of the two detected forms of *Z. v. vivipara* on the southern coast of the Baltic Sea. Their distribution in the Kaliningrad oblast' has been established and some differences in their biotopic characters have been noted. The morphology of sex chromosomes can be evidently used both to identify specimens as well as to recognize subspecies boundaries in *Z. vivipara*. To date a mosaic or sympatric distribution of the above forms is not observed in this region, however a parapatric zone is for the first time determined. There is no cytogenetic indication of introgression in specimens from the localities close to their chromosome border. The chromosome rearrangements, in particular in W-sex chromosome, accompany the form-formation, subspeciation and colonization processes in the species. These data suggest a higher taxonomic status of the western and the Russian forms of *Z. v. vivipara*.

Our results support the hypothesis that some region in the Baltic basin may be a zone of secondary contact between two chromosomal forms of the species and indicate that the parapatric zone between them is located in the central part of the Kaliningrad Region. The available chromosome data allow us to assume that during the postglacial time populations of *Z. vivipara* belonging to the western form of *Z. v. vivipara* have been colonizing the Kaliningrad Region from the West and the South-West and those corresponding to the Russian form of *Z. v. vivipara*, from the East and the South-East.

Keywords: *Zootoca vivipara*; karyotype; sex chromosomes; subspeciation; colonization.

INTRODUCTION

The wide-ranged Eurasian common lizard *Zootoca vivipara* (Jacquin, 1787) is characterized by high geographical variability in the features of its genome, karyotype, type of sex chromosomes, and cytogenetic structure of chromosomes. Several populations markedly differing in their mtDNA (haplotypes), karyotypes, and sex chromosomes have been discovered in Europe (Chevalier et al., 1979; Kupriyanova, 1990; 1997; Kupriyanova and Böhme, 1997; Odierna et al., 1993; 1998; 2001; Heulin et al., 1999; Mayer et al., 2000). Finally, a subspecies *Z. v. carniolica* and six chromosomal forms of nominative subspecies *Z. v. vivipara* have been recognized on the basis of these genomes and cytogenetic characters within of tree groups of karyotypes (see Table 1). One of these chromosomal forms, namely Pyrenean form 1 of *Z. v. vivipara*, has been recently described as a new subspecies *Z. vivipara louislantzi* ssp. nov. — on the basis of the combination of different features, including several chromosome and genome characters (Arribas, 2009).

In addition, these forms and subspecies appeared to differ in the mode of their reproduction. For example, *Z. v. carniolica*, *Z. v. louislantzi* and the Pyrenean form 2 of *Z. v. vivipara* are characterized by primitive oviparous reproductive mode whereas other subspecies and forms, by advanced viviparous mode (see Table 1). The hypoth-

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esis of multiple origin of viviparity in the species has been recently supported by modern genome and chromosomal researches (Odierna et al., 2004; Kupriyanova et al., 2005b; 2006; Surget-Groba et al., 2006).

Eventually, it has been shown that *Z. vivipara* represents a polytypic species including morphologically weakly differentiated subspecies and chromosomal forms with uncertain taxonomic status. It becomes clear that morphologically rather similar specimens from numerous populations of *Z. vivipara* along its vast range in Europe and Asia can be recognized on the basis of combined analysis of their genome or cytogenetic markers and reproductive mode.

The five chromosomal forms and several subspecies discovered have distinct distribution range and often mosaic distribution in western and central Europe. Some of them inhabit small areas, others are relict and rare within one country. Their high karyotype diversity has been observed in Hungary (Puky et al., 2004) and in Austria (three viviparous chromosomal forms of *Z. v. vivipara* and oviparous *Z. v. carniolica*) (Kupriyanova et al., 2006). All detected forms and subspecies appeared to have allopatric and parapatric distributions in these countries. Moreover, no indication of introgression in specimens from the chromosomal and haplotype borders has been observed.

Based on karyological data of *Z. vivipara* from eastern Europe, one viviparous chromosomal form, namely the Russian form of *Z. v. vivipara*, has been revealed in different European parts of Russia as well as in the northern and eastern parts of the Gulf of Finland (Finland, Estonia) in the region of Baltic Sea (Kupriyanova, 1990; Kupriyanova et al., 1995; 2005 a), while the viviparous western form of *Z. v. vivipara* has been found among *Z. vivipara* from the southern coast of the Baltic Sea (Denmark) (Kupriyanova, 1997). Therefore this part of Baltic Sea basin might be a zone of secondary contact of different forms of *Z. v. vivipara* (Kupriyanova, 1997) and a search of hybrid zone and hybrids between them would be of special interest for understanding of the level of reproductive isolation and of the role of chromosomes, in particular, of sex chromosomes in the process of subspeciation as well as for clarifying of their subspecific status although more studies would be needed to confirm this.

The aim of the paper is to apply a combined analysis to *Z. vivipara* from five geographically separate localities of the western region in Russia from the southern coast of the Baltic Sea. We studied karyotype of *Z. vivipara*; identified specimens and clarified the geographical distribution of different chromosomal forms of *Z. v. vivipara*; described their biotopic characters; verified the hypothesis about a contact zone; established the characteristics of this zone and suggested a possible colonizing of the region.

MATERIAL AND METHODS

25 specimens from five localities in the western part of Russia (Kaliningrad oblast') situated in the southern

TABLE 1. Known Subspecies and Forms of *Zootoca vivipara* in Europe: Reproductive Mode, Karyotype Characters, Sex Chromosomes, and Distribution

Subspecies and forms	Reproductive mode	Female diploid number	Type of sex chromosomes	Morphology of W-sex chromosome	Distribution
			I group of karyotypes		
Z. v. carniolica	Oviparous	36	Zw	m microchromosome	Southern-central Europe
Z. v. vivipara (the Hungarian form)	Viviparous	36	Zw	m microchromosome	Central Europe
			II group of karyotypes		
Z. v. louislantzi ssp. nov. (formerly *Z. v. vivipara*, Pyrenean form 1)	Oviparous	35	Z_1Z_2W	ST Subtelocentric macrochromosome	Western Europe: Western Pyrenees, Aquitania
Z. v. vivipara (Pyrenean form 2)	Oviparous	35	Z_1Z_2W	A/ST acrocentric/subtelocentric macrochromosome	Western Europe: Eastern Pyrenees
Z. v. vivipara (the Austrian form, the type of locality)	Viviparous	35	Z_1Z_2W	ST subtelocentric macrochromosome	Central Europe
Z. v. vivipara (the Russian form)	Viviparous	35	Z_1Z_2W	A/ST acrocentric/subtelocentric macrochromosome	Eastern Europe and Fennoscandia
			III group of karyotypes		
Z. v. vivipara (the western form) and *Z. v. pannonica*	Viviparous	35	Z_1Z_2W	SV submetacentric macrochromosome	Western, Central and Eastern Europe, Scandinavia

coast of the Baltic Sea were collected in May – July 2008 and in July 2009.

Population 1. Two females were collected about 10 km north of Svetlyi city, western part of Kaliningrad oblast' (54°45′ N 20°07′ E). Population 2. Four females were collected near the city Bagrationovsk, about 35 km to the south-east from Kaliningrad, southern part of the Kaliningrad oblast', near Polish-Russian border (54°24′ N 20°37′ E). Population 3. Six females and one male were collected near the village Pushkarevo, about 70 km to the east from Kaliningrad, central part of the Kaliningrad oblast' (54°37′ N 21°24′ E) and an additional site in this locality — three females were collected in 7 km to the north-east from the village. Population 4. Five females and two males were collected near the village Dolzhanskoe, about 100 km north-east of Kaliningrad, north-eastern part of the Kaliningrad oblast', near Russian-Lithuanian border (55°03′ N 22°18′ E). Population 5. Two females were collected around Chernyakhovsk city, central part of the region (54°03′ N 21°42′ (38) E, see Fig. 3).

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 of body weight) and then with 0.05% colchicines (0.1 ml/5 g of body weight) 1 h before sacrificing animal. The slides were stained for 10 min with a 5% Giemsa solution in pH 7 phosphate buffer.

To observe the mode of reproduction fifteen pregnant females were kept in a terrarium during June and July up to hatching.

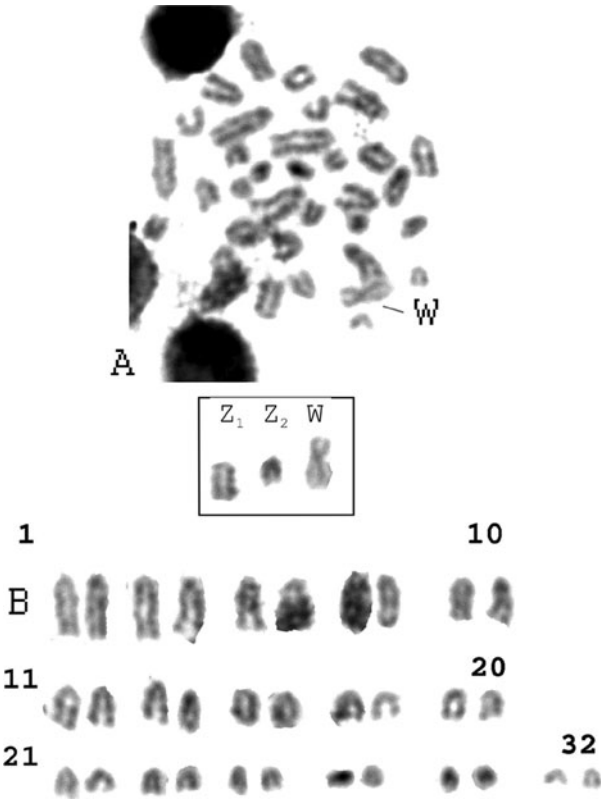

Fig. 1. A. Metaphase of blood cell of *Zootoca vivipara* female from population 3. $2n = 35$, 34 acrocentric macrochromosomes and 1 submetacentric macrochromosome: $2n = 35$ M: 34A + 1SV. Arrow points to the W-sex chromosome. **B.** Karyotype. According to karyotype markers this female belongs to the western form of *Z. v. vivipara*. $2n = 35$: 32 A + Z_1Z_2W with the W-sex submetacentric macrochromosome (SV). In the frame — Z_1Z_2W sex chromosomes.

RESULTS AND DISCUSSION

Reproductive modality, karyotype and identification. The observations for fifteen pregnant females of *Z. vivipara* studied in the terrarium and in nature have shown that they all were viviparous.

Chromosomal analysis has revealed that the males have typical 36 acrocentric (A) macrochromosomes ($2n = 36$ A), whereas females from three geographically separate localities 1, 2, and from two collection sites in the locality 3 have $2n = 35{:}34$ acrocentric (A) macrochromosomes (M) and one biarmed submetacentric macrochromosome (SV). Thus, these females show the Z_1Z_2W type with the W-sex submetacentric macrochromosome (SV), which is well known for *Z. vivipara* (Fig. 1). Neither inter-population chromosome variability, nor intra-population one (mosaic or aneuploid cells and other disturbances in chromosome segregation) were observed. It follows from the above that these specimens from these three new localities studied correspond to one of the known chromosomal forms of the species, namely to the viviparous western form of *Z. v. vivipara*.

Karyotype of females of *Z. vivipara* from localities 4 and 5 contains the same number of chromosomes $2n = 35{:}34$ acrocentric (A) macrochromosomes (M) and one acrocentric/subtelocentric macrochromosome (A/ST). Thus, these females show the Z_1Z_2W type with W-sex macrochromosome. However in their karyotype the W-sex chromosome is represented by the uniarmed acrocentric/subtelocentric macrochromosome (A/ST). No mosaic cells, aneuploidy or other disturbances in chromosome segregation have been also found (Fig. 2). Thus, according to chromosomal analysis the specimens of *Z. vivipara* from populations 4 and 5 belong to different chromosomal form of the species, namely to the viviparous Russian form of *Z. v. vivipara*.

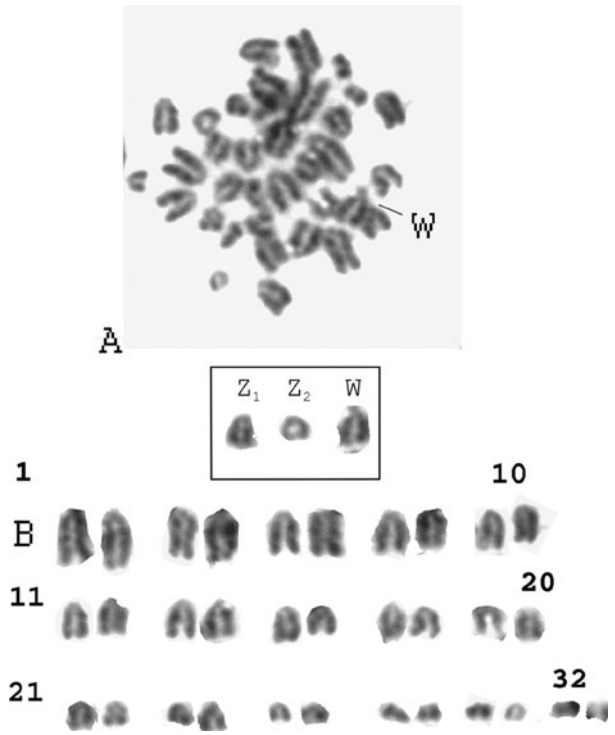

Fig. 2. A. Metaphase of blood cell of *Zootoca vivipara* female from population 4. $2n = 35$, 34 acrocentric macrochromosomes and 1 acrocentric/subtelocentric macrochromosome: $2n = 35$ M: 34 A + 1 A/ST. Arrow points to the W-sex chromosome. **B.** Karyotype. According to karyotype markers this female belongs to the Russian form of *Z. v. vivipara*. $2n = 35$: 32 A + Z_1Z_2W with the W-sex acrocentric/subtelocentric macrochromosome (A/ST). In the frame — Z_1Z_2W sex chromosomes.

Fig. 3. The distribution of *Zootoca vivipara* in the Kaliningrad oblast' based on its karyotypes: circle mark, the viviparous western form of *Z. v. vivipara*; square mark, the viviparous Russian form of *Z. v. vivipara*.

Distribution and biotopic characters. From presented chromosomal data three new localities of the western form of *Z. v. vivipara*, rare for Russia are found. At present, including lizards previously studied, altogether seven localities of this form have been detected, with two points near Kaliningrad (Kupriyanova, 2004; Kupriyanova et al., 2007). Six of them were from western and south-western parts and one — from the central part of the Kaliningrad oblast' (Fig. 3).

Different, the Russian form was for the first time revealed by us in the only locality in the central part of this region (Kupriyanova et al., 2007). Here we report a finding a new collection site in the locality 5 for this form. Additionally, we have detected the second locality of this form of *Z. v. vivipara* (the locality 4) in the north-eastern part of the region (Fig. 3).

At present, these two forms of *Z. v. vivipara* from geographical separate populations of the Kaliningrad oblast' (on the southern coast of the Baltic Sea) inhabit known western and eastern borders of distribution ranges of these forms, the margin of their distribution areas. These forms live relatively close to each other in the central part of the Kaliningrad oblast', the distance between the localities 3 and 5 is around 15 km (Fig. 3).

In addition, some differences in the biotopic characters of these forms, inhabiting the Region have been determined. The populations of the western form of *Z. v. vivipara* are met along the railway road in the Pregolya River valley at an elevation of 39.8 m a.s.l. Population 3 as well as populations 1 and 2 of this form occur in alder-spruce-ash-birch forest (*Alnus incana* Willd, *Picea excelsa* Link, *Fraxinus excelsior* L., *Betula pubescens* Ehrh.) on country roads of such type of forest and in edges of the forest on wet *Sphagnum* peat (*Pseudoscleropodium purum*, *Rhytidiadelphus squarvosus*) and also including shrubbery areas (*Vaccinium vitis-idaea*) and in man-made gardening area. In contrast, both populations of the Russian form of *Z. v. vivipara* (numbers 4 and 5) have been revealed in the meadows with low herbaceous vegetation, on warm and bush sides of country roads. The isolated population 4 lives in a small meadow in forestry area. The area of this meadow is 1 × 1.5 km, mostly with low herbaceous vegetation. No specimens occur in the forest for 15 km around. The population 4 may need protection. A detailed study has been now started.

Moreover, some differences in several cytogenetic and molecular characters of the W-sex chromosomes of these two forms have been observed (Kupriyanova et al., in press). The data indicate that significant reorganization of these chromosomes has occurred. Further karyotype investigations of these two and other chromosomal forms and subspecies of *Z. vivipara* using some modern molecular-cytogenetic techniques are needed.

In conclusion, our chromosomal data indicate that two different forms inhabit the Kaliningrad oblast' on the southern coast of the Baltic Sea, the Russian form of *Z. v.*

vivipara and the western form of *Z. v. vivipara*. Thus, our results confirm the hypothesis (Kupriyanova, 1997) that this part of the Baltic Sea represents a zone of secondary contact between two forms. Neither their mosaic distribution nor sympatry have been observed. To date, these forms appeared to have an allopatric distribution and in one case they have a parapatric distribution, the latter region can be considered as a parapatric zone. This zone is for the first time determined in the central part of the Kaliningrad oblast'. Studying of more samples from the sites between these localities may provide additional information about a hybridization event. At present, our analysis failed to detect any variability in the W-sex chromosome or chromosome disturbances in specimens from the localities of this zone. Therefore there is currently no chromosome indication of introgression in specimens from the zone. This could indicate that both forms deserve subspecific status.

The present and available chromosome data on *Z. vivipara* evidently demonstrate that chromosome rearrangements, in particular in W-sex chromosome accompany the form-formation, subspeciation and colonization processes. During the postglacial time populations of *Z. vivipara* belonging to the western form of *Z. v. vivipara* have been colonizing this region of the Baltic Sea from the West and the South-West whereas those corresponding to the Russian form of *Z. v. vivipara* from the East and the South-East.

Our investigations are still in progress. Future combined researches (cytogenetic, mitochondrial and nuclear DNA, ecological and morphometric analyses) will refine the distribution of different forms and subspecies, clarify their taxonomic status and answer to the questions about the mode of subspeciation and speciation, a possible role of chromosomes, in particular, of sex chromosomes in these processes, about the center(s) of their origin, refugium(a), (re)colonization as well as those about some rare populations and their protection.

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