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Karyotype diversity of the Eurasian lizard *Zootoca vivipara* (Jacquin, 1787) from Central Europe and the evolution of viviparity

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Abstract. We sampled seven populations of *Zootoca vivipara* from Central Europe (6 Austrian, 1 German) and found several chromosomal forms inhabiting this region: (1) oviparous *Z.v. carniolica*, (2) ovoviviparous form from Hungary and southern Austria with the same karyotypic characters, (3) ovoviviparous western form, and (4) ovoviviparous form in the NE Alps including the type locality, which is caryologically similar to the NE Austrian Pannonian lowland populations and the ovoviviparous Russian form. Austria is characterized by high chromosomal diversity in *Z. vivipara*, housing 3 different ovoviviparous chromosomal forms plus an oviparous one. Together with other data sets, our results corroborate the hypothesis of a multiple origin of viviparity in this species.

Introduction

The widely distributed Eurasian lizard *Zootoca vivipara* (Jacquin, 1787) (Reptilia, Lacertidae) is represented in Europe by a mosaical array of populations diverging in karyological and molecular features as well as in reproduction mode. Now it becomes clear that *Z. vivipara* represents a complex including several forms and subspecies. All of them are morphologically only weakly differentiated. However they differ in the mode of reproduction and in several mitochondrial DNA and karyotype features (Heulin et al. 1993, 1999; Kupriyanova 1990, 2004; Kupriyanova & Böhme 1997; Mayer et al. 2000; Odierna et al. 1993; 1998; 2001; Surget-Groba et al. 2001). In the karyotype markers they differ in the female diploid number (2n= 36; 2n=35), in the type of the female sex chromosomes (ZW, Z_1 Z_2 W), as well as in size, constitution and morphology of the W chromosome (w- acrocentric microchromosome, W- macrochromosome: A/ST, A- acrocentric, ST- subtelocentric; SV- submetacentric). At present at least four different karyotypes have been described from females of ovoviviparous *Z. vivipara* from geographically distant populations in Europe. There are two karyologically different oviparous forms, one from the Pyrenean region in western Europe with 2n=35, Z_1 Z_2 W (the Pyrenean form) and another one, the subspecies *Z. v. carniolica*, with 2n=36, Zw recorded from some localities in southern-central Europe. The taxonomic status of these distinctive chromosomal forms with parapatric or allopatric distributions remains uncertain. Some of them inhabit only small areas, others are rare in a country and therefore may need protection there. High genomic diversity has been detected in central Europe (Mayer et. al. 2000; Puky et al. 2004; Surget-Groba et al. 2006; Mayer et al. in prep.) but only few chromosomal data are available.

The objectives of this research were (1) to study the karyotypes of some ovoviviparous and oviparous populations from Austria (including the terra typica of *Z. vivipara*) and Germany, (2) to compare their features with those of chromosomal forms already described and (3) to compare the data with available mtDNA data.

Materials and methods

14 specimens from six localities in Austria and two specimens from one locality in Germany were collected (see Tab. 1). According to mitochondrial DNA data these specimens are representatives of four mitochondrial haploclades (Mayer et al. in prep.). Some of these localities are close to a haploclade border (Fig 1).

Chromosomes were obtained according to the scraping and air-drying method (Olmo et al. 1987; Odierna et al. 1993) from intestine, blood and lung tissues. Specimens were injected with phytogemoglutinin M (Difco) and then with colchicine. The slides were stained for 10 min. with 5% Giemsa solution in phosphate buffer pH 7. C-banding of the chromosomes was carried out according to Sumner (1972).To identify the mode of reproduction in the respective populations, pregnant females were kept in a terrarium up to giving birth to offspring.

Results and discussion

Chromosome sets of all females and two males of *Z. vivipara* (and the mode of their reproduction) from all localities have been studied. Main

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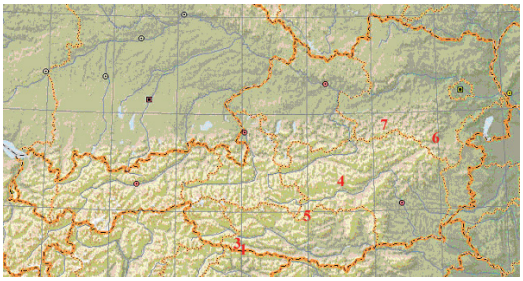
Figure 1. Map showing sampling localities.



characters of karyotypes have been analyzed , i.e. the diploid number (2n), the size (M, m) and the rough morphology of chromosomes, the number of chromosomal arms (NF), number and system of sex chromosomes (ZW; Z_1 Z_2 W), and the morphology of W sex chromosomes (A/ST, SV, a) (Tab. 2). Amount and localization of C-bands in W sex chromosomes of specimens from the terra typica of *Z. vivipara* have been studied for the first time.

Males possessed 36 acrocentric chromosomes, a karyotype correlating with male karyotypes of all chromosomal forms of *Z. vivipara* investigated so far. Female samples differed in karyotype markers and/or in the mode of reproduction. These populations appear to correspond to several already described chromosomal forms in different regions of Europe.

The karyotype of females of *Z. vivipara* from the Klein-Kordin Alm, southern-most Austria, (population 1; "western" haploclade) and from Gänsehals near Maria Laach in Rhineland, western Germany (pop. 2) shows 2n = 35, 34 acrocentric (A) and 1 submetacentric (SV) chromosomes, NF=36, with Z_1 Z_2 W sex chromosome system. The W sex chromosome is a biarmed submetacentric macrochromosome (Fig. 2 a, b). These females are characterized by ovoviviparous reproduction. They belong to the "western" haploclade (like *Z. v. pannonica* from Botany, Slovakia, Mayer et al. in prep.) and correspond karyologically to the known western chromosomal form of *Z. v. vivipara.* This type has a rather wide distribution area mainly in western and central Europe and inhabits many countries (e.g. Bulgaria, Denmark, Germany, the Netherlands, France, Italy, Poland, Sweden, Switzerland). Recently it has been found in the western region of Russia, too (Kupriyanova 2004). This paper reports the first record of the western chromosomal form in Austria.

The karyotype of females from the Strainiger Alm, southern Austria (pop. 3; haploclade "south") shows

Taxon	Samples	Haplotype	Locality
Z. v. vivipara	1♀/1♂ population 1	Type "west"	Klein-Kordin Alm, about 20 km W of the village Hermagor, Carinthia, Carnian Alps, near the Austrian-Italian border
Z. v. vivipara	2♀ population 2		Gänsehals near Maria Laach in Rhineland, western Germany
Z. v. carniolica	2 ♀ population 3	Type "south"	Straniger-Alm, about 100 m below Klein-Kordin Alm, Carnian Alps, near the Austrian-Italian border
Z. v. vivipara	2♀ population 4.	Type "east"	Neunkirchner Hütte, about 20 km N of the village Oberwölz, Styria, Niedere Tauern
	2 ♀ population 5		Turracher Höhe, about 30 km N of Villach, Styrian-Carinthian border, Gurktaler Alpen
Z. v. vivipara	3♀/1♂ population 6	Type "north"	Schneeberg, about 50 km S of Vienna, northeastern Calcareous Alps, terra typica
	2 ♀ population 7		Ötscher region, about 100 km SW of Vienna, northeastern Calcareous Alps

Table 1. Samples and localities of *Z. vivipara* (haplotype is given according to Mayer et al. (in prep.).

Table 2. Results of analyses of *Z. vivipara* from central Europe

Mitochondrial haplotype	2n/♀	System of sex chromosomes	Morphology of W chromosome	Mode of reproduction	Chromosomal form
type "west" pop. 1, 2	35	$Z_1 Z_2$ W	SV	Ovoviviparous	*Z. v. vivipara,* western form
type "south" pop. 3	36	Zw	m, a	Oviparous	*Z .v. carniolica*
type "east" pop. 4, 5	36	Zw	m, a	Ovoviviparous	*Z. v. vivipara,* Hungarian form
type "north" pop. 6, 7	35	$Z_1 Z_2$ W	ST/A	Ovoviviparous	*Z. v. vivipara,* similar to Russian form

2n=36 acrocentric (A) chromosomes, (NF=36), with ZW system of sex chromosomes. The W sex chromosome is an unpaired acrocentric (a) microchromosome (m) (Fig. 2 c). These females of the "southern" haploclade belong to the oviparous subspecies *Z. v. carniolica.* (Mayer et al. 2000). According to the comparative analysis these specimens of the "southern" haploclade correspond to the oviparous subspecies *Z. v. carniolica.* This subspecies has a rather small distribution range in the southern part of central Europe: northern Italy, Slovenia, northwestern Croatia and southern Austria.

The karyotype of females from the Neunkirchner Hütte (pop. 4; haploclade "east") and Turracher Höhe (pop. 5; haploclade "east") in southern central Austria has also shown 2n=36 acrocentric (A) chromosomes, NF=36, with a ZW system of sex chromosomes. Like in *Z. v. carniolica* its W sex chromosome is an unpaired acrocentric (a) microchromosome (m) (Fig. 2 d). However, these females are characterized by ovoviviparous reproduction. We should stress that in spite of the similar karyotype structure, *Z. v. carniolica* (pop. 3) and this clade of *Z. v. vivipara* (pop. 4 and 5) belong to different haploclades (types "south" and "east", respectively) and are characterized by different reproduction mode. According to combined analyses (karyotype features: 2n=36, NF=36, Zw, w-m and viviparous reproduction) these specimens from southern central Austria correspond to those recently described from one relict population in central Hungary (Odierna et al. 2004; Puky et al. 2004). The present paper reports the second record of this form (Hungarian form).

The populations from Schneeberg (pop. 6) and from the Ötscher region (pop. 7) in north-eastern Austria studied for the first time are representatives of the mitochondrial type "north" (Tab. 1), which include not only the terra typica of the species but also Austrian viviparous populations usually assigned to the subspecies *Z. v. pannonica* (Mayer et al. in prep.). The female karyotype has shown 2n=35, 34 acrocentric (A) chromosomes and one uniarmed subtelocentric/acrocentric (ST/A) macrochromosome (M), NF=35, a $Z_1 Z_2$ W sex chromosome system, where the W sex chromosome is unpaired ST/A (Fig. 3 a). The analysis discovered high similarities by several karyotype markers (2n=35, $Z_1 Z_2$ W, W-ST/A) between these samples and those of Austrian so-called *Z. v. pannonica* (viviparous), the Pyrenean form (oviparous, Portalet population) and the viviparous Russian form of *Z. v. vivipara*. The latter was discovered by karyological methods in several geographically very distant populations across European and Asian Russia (Kupriyanova 1986), the Transcarpathian region of Ukraine (Kupriyanova, 1990), eastern Hungary (Odierna et al., 2004; Puky et al., 2004), in central Estonia (Kupriyanova, 1997) and eastern Finland (Kupriyanova et al., 2005a). Comparative C-banding analysis of W chromosome of representatives of the terra typica and the Russian form exhibits some similarities in the localization of constitutive heterochromatin blocks. Most of the autosomes and the subtelocentric W chromosome

possess conspicuous centromeric and telomeric C-bands. Additionally, the W sex chromosome has one remarkable interstitial C-band (Fig. 2 b). Nevertheless, there are some differences in the amount of heterochromatin in centromeric C-blocks - namely prominent centromeric and interstitial C-bands in the terra typica form but smaller bands in the Russian form (Fig. 5 c). Presently we have no information from some additional chromosomal markers in order to characterize more precisely and better to differentiate the specimens from Austria. Hence for comparison we still need additional information. According to mtDNA analysis these viviparous populations belong to different haploclades: the former to the haploclade "north" whereas the latter one to the "Russian" haploclade (Mayer et al. in prep.). Future cytogenetical and molecular researches may provide us with valuable information to resolve the relationships of these chromosomal forms belonging to different haploclades. Extensive interpopulation karyotype analyses as well as detailed investigations of the chromosome structure may also help in solving several open questions concerning the centres of formation and differentiation of the forms and their glacial refugia, postglacial colonizations and the evolution of viviparity. From just presented and available chromosomal and cytogenetical data the situation in *Z. vivipara* is more complex than we thought earlier. Combined analyses of chromosomal, reproduction and mitochondrial DNA data support the hypothesis that viviparity arose more than once

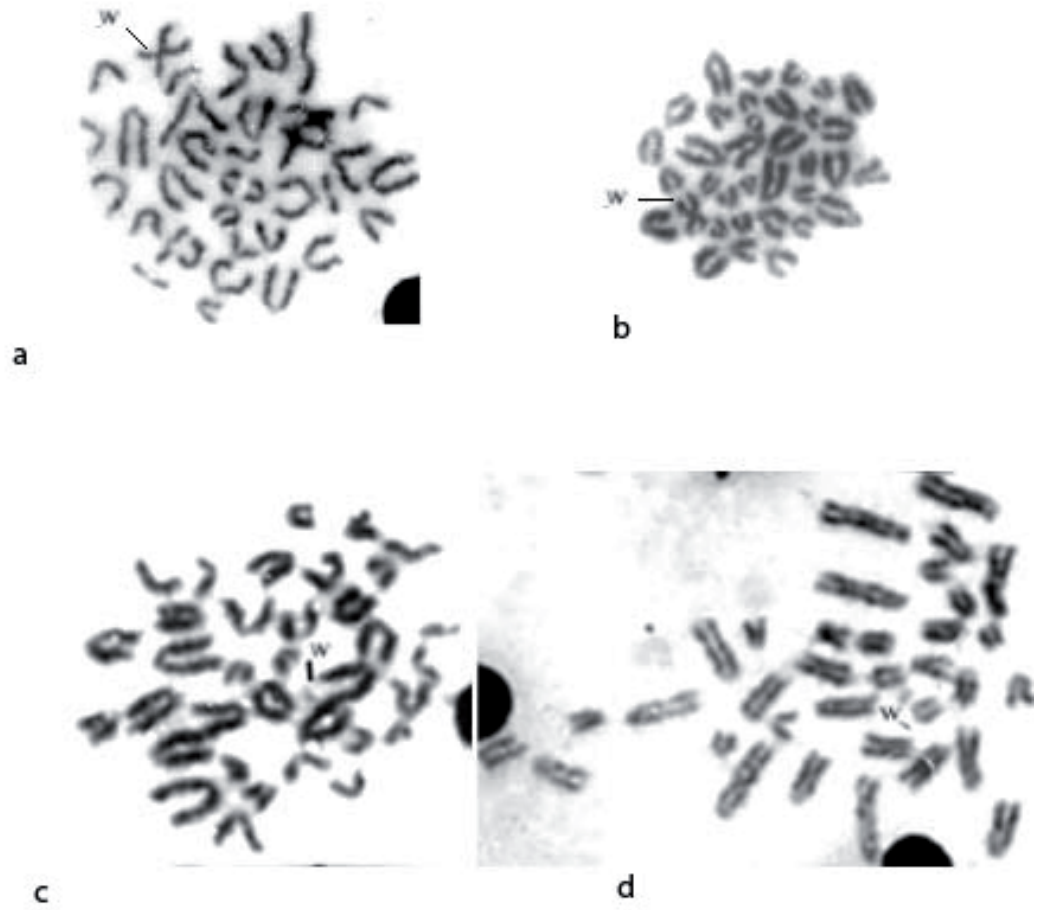


Figure 2. Giemsa stained female metaphase plates of *Zootoca vivipara.* a, b - specimens from populations 1 and 2. 2n = 35, Z_1 Z_2 W, W- SV: *Z. v. vivipara*, western form; c – specimen from population 3, 2n = 36, Zw, w – m, a: *Z .v. carniolica;* d - specimen from population 4, 2n = 36, Zw, w –m, a: *Z. v. vivipara,* Hungarian form. Arrows point to W - sex-chromosome.

independently in various populations of *Z. vivipara* (Odierna et al. 2004; Kupriyanova et al. 2005b; Surget-Groba et al. 2006). Probably viviparity arose at least in oviparous populations with females possessing 2n=36, Zw (like the oviparous *Z. v. carniolica)* and in another oviparous population with females displaying 2n=35, Z_1 Z_2 W (like the oviparous Pyrenean form of *Z. v. vivipara)*. The molecular data do not rule out a possibility of reversal back to oviparity (Surget-Groba et al. 2006). The taxonomic status of all forms of *Z. vivipara* characterized by different karyotypes, reproduction mode and mitochondrial DNA sequences needs clarification. Presently there are no indications of introgression in specimens from localities close to borders of different mitochondrial or chromosomal forms.

Conclusions

As a result of karyological investigations of seven populations of *Z. vivipara* from Central Europe we found that several chromosomal forms of *Z. vivipara* inhabit this region. They are (1) the oviparous *Z. v. carniolica* and (2) an ovoviviparous form of *Z. v. vivipara* (Hungarian form) with the same karyotype characters, (2n=36, NF=36, Zw, w-m), which is probably endemic in southern Austria and parts of Hungary (3) an ovoviviparous form (the western form) of *Z. v. vivipara* with 2n=35, NF=36, Z_1 Z_2 W, W-SV and (4) an additional ovoviviparous form in the northeastern-most Alps (including the terra typica population) karyologically rather similar to so-called *Z. v. pannonica* (ovoviviparous) from the northeastern Austrian lowlands and to the ovoviviparous Russian form with 2n=35, NF=35, Z_1 Z_2 W, W–ST/A, the W sex chromosome with three remarkable C-bands.

Finally it has been demonstrated that (i) central Europe (Austria) is characterized by high diversity of chromosomal forms of *Z. vivipara*, (ii) three different chromosomal forms of *Z. v. vivipara* occur among ovoviviparous populations (iii) an additional ovoviviparous population group of *Z. v. vivipara* (the Hungarian form) lives in Hungary and parts of Austria in restricted areas and may need protection, at least in Hungary, (iv) there is some correlation

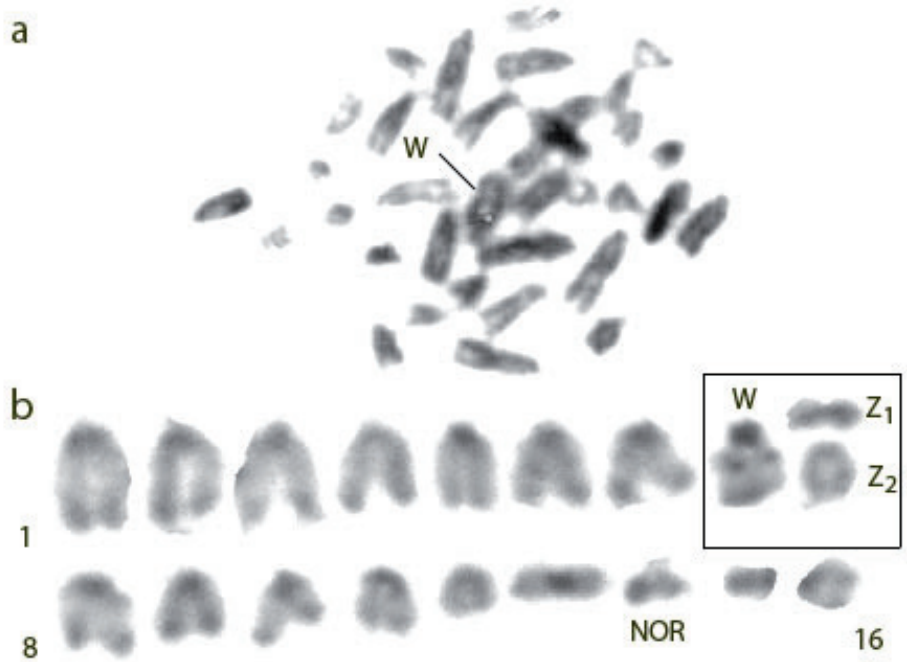


Figure 3. Giemsa stained female metaphase plate (a) and C-banded karyotype (b) of *Z. v. vivipara* (population 7) 2n = 35, Z_1 Z_2 W, W- ST/A. Arrow points to W-sex chromosome

between chromosomal and mtDNA data, and (v) comparative analyses of chromosomal, molecular and reproductive data from central Europe support a previous hypothesis (Odierna et al. 2004; Kupriyanova et al. 2005b; Surget-Groba et al. 2006) that viviparity arose more than once in different populations of the species.

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