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Karyotype dispersal of the common lizard *Zootoca vivipara* (Lichtenstein, 1823) in eastern and northeastern Fennoscandia

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Abstract

The wide-ranging Eurasian common lizard *Zootoca vivipara* (Lichtenstein, 1823) is remarkably uniform morphologically but highly varied in its karyotype. Previous studies have revealed two distinctly different chromosomal forms of *Z. v. vivipara* in the Baltic basin. Moreover, a zone of secondary contact between these forms has been localized on the southern Baltic Sea seashore. Intraspecific karyotype diversity for *Z. vivipara* and new zones of secondary contact have recently been suggested for other parts of the Baltic Sea seashore. We studied the karyotype of *Z. vivipara* in central, western and northern parts of Finland. All the individuals karyotyped represented the Russian form of *Z. v. vivipara* that differs from the western form of the subspecies located at the southern and western Baltic Sea seashore. Together with previous data sets, our results suggest intraspecific karyotype diversity in the northern and northwestern parts of Fennoscandia. The results give support to the hypothesis of *Z. vivipara*’s re-colonization of the Baltic Sea basin. Moreover, the results support the previous observations of Voipio (1961, 1968 and 1969) who has reported variability in the shield pattern of *Z. vivipara* in the same region.

Introduction

The widely-ranging Eurasian common lizard *Zootoca vivipara* (Lichtenstein 1823) (family Lacertidae) is a squamate species with a huge distribution range from western Europe (the Pyrenees) throughout central, eastern and northern Europe up to eastern Asia (the Russian
Far East, islands Sakhalin and Kunashir and northern Japan). The species is characterized by viviparous and oviparous reproduction in different populations (Brana & Bea 1987) and substantial geographic variation in body size and reproductive output (Horváthová et al. 2013). Despite such special characteristics *Z. vivipara* is remarkably uniform morphologically but polymorphic in its haplotype and karyotype. The species has 1) different diploid numbers: $2n = 36/36$ in both sexes or $2n = 35$ in female and $2n = 36$ in male; 2) different size of female sex chromosomes: $w$ microchromosome (m) or $W$ macrochromosome (M); 3) different systems of sex chromosomes: $Z_w$ in female and $ZZ$ in male or $Z_1Z_2W$ in female and $Z'_1Z'_2Z_2$ in male; 4) different morphology of $w$ and $W$ sex chromosomes: acrocentric (a, A), subtelocentric (ST) or submetacentric (SV) and 5) some differences in cytogenetic and molecular structure of $w$ and $W$ sex chromosomes: heterochromatic amount and some other features (Table 1.).

From all these karyotype characteristics six/seven separate chromosomal forms have been recognized among oviparous and viviparous females from different populations in Europe and in Asia. Among them, two new oviparous subspecies; *Z. v. carniolica* (Mayer, Böhme, Tiedemann & Bischoff 2000) and *Z. v. louislantzi* (Arribas 2009). Two recent studies even suggest that *Z. v. carniolica* may be approaching species status (Lindtke et al. 2010, Cornetti et al. 2014). Subspecies *Z. v. vivipara* may be subdivided into four viviparous chromosomal forms, three of which are closely related, although the taxonomy of the latter is still questionable, they can be easily recognized by their $2n$ and some other karyotype characteristics (Table 1.).

In addition, several molecular and chromosomal studies have discussed the geographical distribution of different haplogroups (Heulin et al. 1999, 2011, Surget-Groba et al. 2001, 2006, Velekei et al. 2014) and chromosomal forms (Kupriyanova 1990, Kupriyanova & Böhme 1997, Kupriyanova et al. 2005, 2006, 2007, Odierna et al. 2001, Puky et al. 2004) of *Z. vivipara*. Chromosomal studies have shown that described subspecies and separate chromosomal forms of *Z. v. vivipara* have their distinct distribution ranges in Europe and in Asia (Table 1). In central Europe, subspecies and forms occur in allopatric, parapatric and sometimes mosaic populations. Some of them appear to inhabit small areas while others are relict and rare within one country. However, the western form of *Z. v. vivipara* and the Russian form of *Z. v. vivipara* occupy a vast territory in Europe and Asia. It has been indicated that the Russian form has in its female karyotype 34 acrocentric (A) chromosomes.
and 1 acrocentric (A) W sex chromosome (35 chromosomes in total). The latter has short arms at some metaphase plates and it is close to subtelocentric (ST). Therefore, it is sometimes indicated as A/ST. Chromosomal formula is: ♀2n = 35: 34A + 1A, where W is A (or A/ST). The western form has in its female karyotype 34 acrocentric (A) chromosomes and 1 submetacentric (SV) W sex chromosome (the same 35 chromosomes in total). Chromosomal formula is: ♀2n = 35: 34A + 1SV, where W is SV. The males of all forms of Z. v. vivipara and of subspecies Z. v. louislantzi have in their karyotype 36 acrocentric chromosomes: ♂2n = 36A with 4 acrocentric Z1,Z1,Z2,Z2 sex chromosomes (Table 1.).

Based on the karyotype (Kupriyanova 1990, 2004, Kupriyanova & Rudi 1990, Kupriyanova & Böhme 1997, Kupriyanova et al. 2007, Odierna et al. 1998, 2001) and the Mt DNA data (Surget-Groba et al. 2006, Velekei et al. 2014), the Russian form has been discovered from the eastern Carpathian throughout Russia up to Sakhalin island and northern Japan, whereas the western form has also been found in the populations in the eastern and western Carpathians as well as in central and in western Europe up to the Pyrenees. So far, the highest karyotype diversity has been discovered among the populations in the Carpathian Basin, due to which a centre of evolution of different chromosomal forms of Z. vivipara has been assumed to occur there (Kupriyanova & Böhme 1997, Odierna et al. 1998). Additionally, from all the available biogeographical and chromosomal data, it can be concluded that the Russian form is the most primitive one, whereas the western form has been derived from it (Kupriyanova 1990, Kupriyanova & Rudi 1990, Odierna et al. 1998).

Based on the karyotype markers, many specimens of western form Z. v. vivipara have been further identified in the western part of the Baltic region (Denmark, north of Germany, south of Sweden), while specimens of Russian form could be found in its eastern part (Estonia, north-west of Russia, south-east of Finland). Therefore, it was predicted that the southern Baltic Sea is a zone of secondary contact between these two chromosomal forms (Kupriyanova 1997).

In previous chromosomal studies, both the western and the Russian form of Z. vivipara have been identified on the limited territory of this seashore, namely the Kaliningrad oblast [Königsberger Gebiet] in western Russia (Kupriyanova et al. 2007, Kupriyanova & Melashchenko 2011). Therefore, intraspecific karyotype diversity in northeastern Europe has been confirmed and a zone of secondary contact between these forms localized. In addition, the previous data by Kupriyanova & Melashchenko (2011) predicts more intraspecific
karyotype diversity and several new zones of secondary contact in Kaliningrad oblast and in other parts of the southern Baltic Sea seashore. This has been confirmed by a karyological study of *Z. vivipara* where both forms were discovered in Poland for the first time in 2012 by Kupriyanova and Böhme (2012) (see Fig. 1). Moreover, two new zones of their secondary contact with allopatric and in one case with a parapatric distribution in Kaliningrad oblast were found in 2014 by Kupriyanova and Melashchenko (2015).

Thus, the geographically distinct distribution of both forms has been demonstrated and the border of their distribution area in this part of northeastern Europe has been verified. The previous data sets suggest that during the postglacial time, populations of *Z. vivipara* belonging to the western form of *Z. v. vivipara* have been re-colonizing the Kaliningrad region from the west and south-west, and those belonging to the Russian form of *Z. v. vivipara* from the east and south-east. Moreover, the previous data enables us to predict karyotype diversity of *Z. vivipara* and some new zone(s) of secondary contact between the two forms in other parts of the Baltic basin as well as a trend of re-colonization of the Baltic region by *Z. vivipara* during the post-glacial period (Kupriynova & Melashchenko 2011, 2014, Kupriyanova & Böhme 2012).

To test these predictions, we focused on the diagnostics of morphologically uniform specimens of *Z. vivipara* from populations along the eastern and northern sides of Gulf of Bothnia. We collected 33 specimens of *Z. vivipara*, obtained chromosomes and studied several previously listed karyotype markers to evaluate the karyotype diversity of *Z. vivipara* on the eastern and northern coasts of the Baltic Sea. The data allowed us to 1) define the karyotype of *Z. vivipara* from central and western parts of Finland as well as from a southern part of northern Finland; 2) identify the specimens; 3) verify a border of distribution of different forms of *Z. vivipara* on studied regions and 4) test a hypothesis of re-colonization by specimens of *Z. vivipara* of this part of Fennoscandia.

**Materials and Methods**

25 specimens from nine geographically distinct localities from central, western and northern Finland were collected and analyzed in May – June 2011 and 2012 (Table 2; Fig. 1). In addition, we analyzed eight individuals (6 females and 2 males) from an enclosure population originating from several natural populations in central Finland and located at Konnevesi Research Station of the University of Jyväskylä (locality 1 in Table 2).
The chromosomes were obtained according to the scraping and air-drying method from intestinal epithelial and lung cells as well as from the germinal lamina (i.e. the ovarian area where the earliest stages of oogenesis occur) with using 0.05 % colchicines (Odierna et al., 1993). In a subset of the samples, we used a different method where metaphase chromosomes were prepared from whole blood and a short term leucocyte culture in Krea vital Lymphocyte Karyotyping Medium with an addition of 0.1 ml 0.1 % phytohaemagglutinin M (Sigma-Aldrich) per 3.5 ml culture for 24 – 48 h and of 0.1 ml 0.002 % colchicine for 30 min (modification of the method of Moritz 1984, 1987). The slides were stained for 10 min with a 5 % Giemsa solution in pH 7 phosphate buffer. Metaphase plates suitable for chromosome analysis were obtained from all samples studied.

Results and discussion

Chromosomal analysis showed that females of *Z. vivipara* from geographically separate localities 1-10 (Table 2; Fig. 1) have 2n = 35:34 acrocentric (A) macrochromosomes and one acrocentric macrochromosome (A), with short arms at rare metaphase plates, sometimes close to subtelocentric (A/ST). Acrocentric macrochromosome (A) is well known as W sex chromosome to *Z. vivipara* (Fig. 2 a – h). Therefore, chromosomal analysis identified these specimens as the Russian form of *Z. v. vivipara*.

A limited number of specimens (2 - 4 from each locality) does not allow us to assess inter-population or intra-population chromosomal variability (mosaics, polymorphism etc.). Nevertheless, we found for the first time that specimens of the Russian form inhabit the eastern and northern coast of the Baltic Sea (Fig. 1). The data indicates that the Russian form lives in many regions of Finland and in the southern part of northern Sweden. However, an earlier study has identified a western form in the southern and eastern parts of Sweden (Göteborg and Uppsala regions) (Kupriyanova et al. 1995). Moreover, according to molecular (Mt haplotype) data by Surget-Groba et al. (2006) two specimens of a western haplotype (VB haplogroup) have been identified from the south and the central-eastern Sweden (Runsten and Umeå localities) and positioned to western viviparous clade (clade E). At the same time, a specimen west to the border between Sweden and Finland (Kiruna locality) was identified as an eastern haplotype (VU haplogroup) and positioned to an eastern viviparous clade (clade D).
Comparison of molecular phylogenetic trees with karyotype characteristics of different chromosomal forms has demonstrated a good correlation between molecular and chromosomal data (see Kupriyanova 2004, 2013, Kupriyanova et al. 2006). Therefore, it is clear that chromosomal data supports the presence of main branches of the molecular trees of Z. vivipara and shows that their appearance is marked by the chromosomal rearrangements with the forming of several subspecies and separate chromosomal forms. From all these data, we may with confidence say that the specimens belonging to western viviparous clade (clade E, VB haplogroup) should be identified as western chromosomal form of Z. v. vivipara whereas those belonging to eastern viviparous clade (clade D, VU haplogroup) should be identified as Russian chromosomal form. Thus all these data points to the direction that the Russian chromosomal form of the subspecies Z. v. vivipara inhabits north-eastern part of Sweden.

To conclude, our chromosomal data demonstrates that 1) the Russian form of Z. v. vivipara inhabits the central, western and southern parts of northern Finland; 2) these regions are not characterized by karyotype diversity of Z. vivipara; 3) diversity and a zone of secondary contact between two chromosomal forms of Z. v. vivipara may be predicted for other northern parts of Finland as well as for those of Sweden and Norway; 4) the border of the distribution area of the Russian form is located in the north-western and northern Baltic Sea seashore (in the northern parts of Sweden, Finland and/or Norway) and 5) the hypothesis of re-colonization of the area of the Baltic Sea by Z. vivipara is supported.

Regarding the re-colonization hypothesis, our results suggest that during the postglacial time, populations of Z. vivipara belonging to the western form of Z. v. vivipara came to the area from south and south-west whereas those belonging to the Russian form of Z. v. vivipara moved into Fennoscandia from east and south-east. Additionally, the present data is consistent with the previous chromosomal results on the presence of the Russian form both in the southern and eastern parts of Finland and in a neighbouring Karelian Russia territory, near the border between Russia and Finland (Kupriyanova et al. 2005).

Z. vivipara is distributed all over Finland (e.g. Terhivuo 1993) and it should be stressed that our results regarding the chromosomal characteristics correlate with the data of Voipio (1961, 1968, 1969, 1992) reporting variability in the shield pattern of the Z. vivipara in Fennoscandia. Based on these patterns, Voipio pointed out that Z. vivipara populations in southern and central Sweden include specimens with western and central European type of
shields patters whereas specimens in northern Sweden, Finland and Russia (north of the 62° N) show patterns of the eastern type.

To summarize, we emphasize that the identification of specimens of *Z. vivipara* based on their morphology is very difficult and misidentifications may occur. Our results demonstrate the value of chromosome diagnostic of *Z. vivipara* from the geographically distant localities of Fennoscandia. Furthermore, intensive chromosomal studies of specimens from the areas of Finland, in particular those from its northern part, could show a presence of both chromosomal forms of the subspecies *Z. v. vivipara*. New chromosomal data could also give additional information about the karyotype diversity of *Z. vivipara* throughout the area of Fennoscandia and clarify the border of the distribution of the two forms in the region.

From the literature and the data obtained in this study, we predict that the western and Russian chromosomal forms of *Z. v. vivipara* occupy the northern and northwestern regions of Fennoscandia. However, more data from a wider set of localities, in particular from the northern part of Finland, Sweden and Norway, is needed to confirm this prediction as any chromosomal data for these territories is still missing. We would also like to stress that a more detailed study of a secondary contact zone and its characteristic (allopatry, sympatry, parapatry and/or hybrid zone) is needed as *Z. vivipara* may represent a group of cryptic taxa. The taxonomic status of the chromosomal forms *Z. v. vivipara* is still unclear and under discussion in the literature (Kupriyanova 2004, Kupriyanova & Melashchenko 2011). A combination of different types of approaches (chromosomal, molecular, morphometric, life-history and behavioral) would be helpful in evaluating the biodiversity and conservation issues of these unique populations of *Z. vivipara*.

**Acknowledgments**

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**Table 1.** Karyotype characteristics of subspecies and different forms of *Zootoca vivipara* (Lichtenstein 1823) and their distribution in Europe (Size of sex chromosomes: m = microchromosome, M = macrochromosome; Morphology of sex chromosomes a/A = acrocentric, ST = subtelocentric, SV = submetacentric; Mode of reproduction: O = oviparous, V = viviparous). A modification of Table 1. in Kupriyanova (2013).

<table>
<thead>
<tr>
<th>N/N</th>
<th>2n</th>
<th>Sex chromosomes</th>
<th>Mode of reproduction</th>
<th>Localities</th>
<th>Species, subspecies, chromosomal forms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Size/System/Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The first group of karyotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>36A/36: 35A + 1a</td>
<td>m Zw a</td>
<td>O</td>
<td>Central, South-western Europe</td>
<td>Z. vivipara, now Z. v. carniolica</td>
</tr>
<tr>
<td>2.</td>
<td>36A/36: 35A + 1a</td>
<td>m Zw a</td>
<td>V</td>
<td>Central Europe</td>
<td>Z. vivipara, now Z. v. vivipara Hungarian form</td>
</tr>
<tr>
<td>The second group of karyotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>36A/35: 34A + 1A (A/ST)</td>
<td>M Z;Z2W A, A/ST</td>
<td>O</td>
<td>Western Europe, the Pyrenees</td>
<td>Z. v. vivipara Pyrenean form, now Z. v. lousianzti</td>
</tr>
<tr>
<td>5.</td>
<td>36A/35: 34A + 1A</td>
<td>M Z;Z2W A</td>
<td>V</td>
<td>Asia, Eastern Europe, Baltic region</td>
<td>Z. vivipara, now Z. v. vivipara Russian form</td>
</tr>
<tr>
<td>6.</td>
<td>36A/35: 34A + 1SV</td>
<td>M Z;Z2W SV</td>
<td>V</td>
<td>Western, Central Europe, Baltic region</td>
<td>Z. vivipara, now Z. v. vivipara Western form</td>
</tr>
</tbody>
</table>
Table 2. Number and origin of specimens of *Zootoca vivipara* analyzed in this study.

<table>
<thead>
<tr>
<th>Locality number</th>
<th>Number of female specimens</th>
<th>Number of male specimens</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>2</td>
<td>Central Finland (enclosure population)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>Konnevesi 62°36.964' N 26°20.746' E</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0</td>
<td>Vesanka 62°30.277' N 25°558' E</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
<td>Muurame 62°05.159' N 25°36.353' E</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0</td>
<td>Alaveteli 63°42.351' N 23°17.784' E</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0</td>
<td>Kortesjärvi 63°18.836' N 23°14.682' E</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0</td>
<td>Vaasa 63°6.943' N 22°2.803' E</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0</td>
<td>Närpiö 62°31.892' N 21°15.404' E</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0</td>
<td>Kauhajoki 62°18.166' N 22°33.502' E</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0</td>
<td>Tornio 65°54.093' N 24°27.957' E</td>
</tr>
</tbody>
</table>

*Northern Finland (north of the Baltic coast)*
**Figure 1.** The locations sampled in this study presented as the numbered squares that match the coordinates in table 2. The map also shows wider distributions of Russian (■) and western (●) forms.
Figure 2. Giemsa stained metaphase plates of females of *Zootoca vivipara* from: 1. Central Finland (localities 1, 2, 4), 2. Western Finland (localities 5, 6, 7, 9) and 3. Northern Finland (locality 10). Localities refer to table 2. 2n = 34A + 1A (A/ST). Arrows point to acrocentric-(A) (e, f, h) and acro-/subtelocentric (A/ST) (a, b, c, d, g) W sex chromosomes. According to karyotype markers these females belong to the Russian form of *Z. v. vivipara*. 