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CHROMOSOMAL CHANGES AND FORM-FORMATION, SUBSPECIATION IN THE WIDERANGED EUROASIAN SPECIES *Zootoca vivipara* (EVOLUTION, BIOGEOGRAPHY)

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INTRODUCTION

The wideranged Euroasian species *Zootoca vivipara* (family Lacertidae) is a rare species, possessing transpale-arctic distribution from the Pyrenees up to the archipelago of the Sea of Japan. A further very important feature is that *Zootoca vivipara* has two reproductive modes in different populations. Primitive oviparous populations inhabit western Europe (the Pyrenees region and the south-eastern central Europe), whereas advanced viviparous populations occur in the other part of the distribution range.

Karyological investigations of *Zootoca vivipara* from many geographically distant populations have shown that the species is characterized by differences in the diploid number [$2n = 36/36$ in both sexes or 36 (male)/ 35 (female)]; in the system of sex chromosomes (ZW or $Z_1 Z_2 W$) and in the types and structure of W sex chromosome (Kupriyanova, 1990, 1997; Odierna et al., 1993).

Morphological differentiation of the populations is poorly pronounced because only four subspecies are recognized: *Z. v. vivipara*, *Z. v. carniolica*, *Z. v. sakhalinensis* (*nomen nudum*), and *Z. v. pannonica*.

Modern cytogenetical studies have revealed five structures of karyotypes among ovi- and viviparous populations of *Z. vivipara* from different geographic ranges.

KARYOTYPE VARIATIONS

One type is the karyotype for specimens from primitive oviparous populations of new genetically described subspecies *Z. v. carniolica* (Mayer et al., 2000) from Slovenia. $2n = 36A$ (acrocentric) (male)/ $36A$ female, system

of sex chromosomes is ZW, where W is fully heterochromatic micro chromosome (w). All autosomes have tiny centromeric heterochromatic C-bands. W-sex chromosome arose as a result of deletion of a primitive acrocentric macrochromosome W (Odierna et al., 2001). Thus, karyological data obtained have confirmed the status of new subspecies. The karyotype structure is sharply different from that of other oviparous and viviparous populations of *Z. vivipara*.

The next two cytotypes are for specimens from oviparous populations of *Z. v. vivipara* from the Pyrenees region (Kupriyanova and Böhme, 1997; Odierna et al., 1998). $2n = 36A$ (male)/ $35A$ (female), system of sex chromosomes is $Z_1 Z_2 W$. Autosomes and acrocentric macro- W_{1A} or subtelocentric W_{1B} -sex chromosomes have tiny heterochromatic C-bands. Acrocentric macro- W_{1A} chromosome has arisen as a result of tandem fusion of auto- and macro W-sex chromosome ("Pyrenean" form). Its karyotype characteristics in sex- and autosomes suggest a higher rank of this form (Odierna et al., 1998).

Two other karyotype structures were found in specimens of advanced viviparous forms of *Z. v. vivipara* from different localities, the first one from central and eastern Europe and Asia and the second from central and western Europe. The former have the same karyotype structure like that of the "Pyrenean" form. $2n = 36A$ (male)/ $35A$ (female) with $Z_1 Z_2 W$ system of sex chromosomes and acrocentric/subtelocentric type of W_2 -sex chromosome (Kupriyanova, 1990). However, unlike the "Pyrenean" form most of chromosomes, including W_2 -sex chromosome of these specimens possess considerable heterochromatic C-bands ("Russian/eastern" form). Therefore the mechanism of chromosome changes is heterochromatinization events.

One more karyotype was discovered for specimens of advanced viviparous forms of *Z. v. vivipara* from central and western Europe. $2n = 36A$ (male)/ $35A$ (female) with $Z_1 Z_2 W$ system of sex chromosomes and meta-/subtelocentric type of W_3 -sex chromosome (Chevalier et al.,

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1979; Kupriyanova, 1990; Odierna et al., 1993). Metacentric W_3 -sex chromosome arose as a result of pericentric inversion of acrocentric sex chromosome. This karyotype has also intensive heterochromatin C-bands in sex chromosome and autosomes (“western” form).

The mechanisms and steps of chromosomal changes of sex chromosomes in the evolution of *Zootoca vivipara* are as follows: deletion, tandem fusion, heterochromatinization event, and pericentric inversion.

Viviparous specimens of *Z. vivipara* from Sakhalin Island, geographically belonging to *Z. v. sakhalinensis* (*nomen nudum*), differed neither by the karyotype (Kupriyanova and Böhme, 1997) nor by haplotype (Mayer and Böhme, 2000) from the “Russian/eastern” form of *Z. v. vivipara*. Therefore these specimens should be considered as *Z. v. vivipara*.

The karyotype of viviparous specimens of *Z. v. pannonica* from Austria did not also differ from that of the “Russian/eastern” form of *Z. v. vivipara* (Kupriyanova and Böhme, 1997). Recent cytogenetical investigations have revealed one other karyotype structure in the subspecies (Odierna et al., 2004). Therefore the question about validity of *Z. v. pannonica* should be revised.

Sympatry, hybrid or contact zones between different chromosomal forms and subspecies have not been found. From the karyological data all of them appear to have distinct distribution range. However it becomes clear that karyologically *Z. vivipara* constitutes a mosaic of populations.

As a result of cytogenetical research three chromosomal forms of *Z. v. vivipara* have been described. Recently one more new chromosomal form has been discovered (Odierna and Kupriyanova, in press). Thus, *Z. vivipara* represents karyologically a complex, including subspecies *Z. v. carniolica* and several chromosomal forms of *Z. v. vivipara*. These studies have shown that the forms have their own distinct distribution ranges; both primitive oviparous “Pyrenean” form *Z. v. vivipara* and *Z. v. carniolica* are characterized by narrow ranges, whereas both advanced viviparous forms of *Z. v. vivipara* possess wide ranges.

GENETIC VARIATIONS

Analyses for 12S rRNA and cytochrome b genes of *Z. vivipara* have shown geographic variation in their haplotypes (Surget-Groba et al., 2001). Five clusters mainly correlating with the karyotype’s groups have been observed.

From the allozyme analysis a mean genetic distance, e.g., between oviparous and viviparous populations from the Pyrenees region and between the former and those from the Balkanic region are short. Nei’s index between the populations are 0.12 (Bea et al., 1990) and 0.102 (Guillaume et al., 1997). These values do not appear to reach the species rank. The laboratory cross experiments between oviparous and viviparous specimens produced some vital hatchlings and demonstrated incomplete reproductive isolation (Heulin et al, 1989; Arrayaco et al., 1996).

EVOLUTION IN THE COMPLEX

All the characteristics listed give a rare possibility to use the species as a model for studying some general evolutionary and biogeographic questions.

Evidently karyological differentiation in the complex is high. Chromosomal rearrangements accompany the active form-formation and subspeciation processes. Steps and mechanisms of these changes in the evolution of the species have been suggested (Kupriyanova, 1990; 1997; Odierna et al 1993; 1998; 2001; Surget-Groba et al., 2001). These are deletion, tandem fusion, heterochromatinization events, and pericentric inversion. It becomes clear that karyotype features may serve as a good marker for the identification of different populations of *Z. vivipara* in the complex.

All these facts argue the significance of cytogenetical data for the understanding of the evolution, phylogeny and biogeography in the complex. Investigations of new markers of W-sex and autosomes of the species may provide more detailed information on their structure. Karyological and different comparative staining analyses of C-banding/CMA₃/DAPI may elucidate in detail the evolutionary steps and a possible role of chromosomal changes in the process of form-formation and subspeciation.

Therefore this paper presents for the first results of karyotype and cytogenetical analyses of specimens of *Z. vivipara* from three earlier unstudied geographically distant populations. In the paper we discuss the biogeography and evolutionary problems and possible modes of form-formation and subspeciation in the complex.

MATERIAL AND METHODS

Nine lizards of *Z. vivipara* (8 females and 1 male) from the upper part of the Eastern Carpathian Great Ridge (Transcarpathian region, Ukraine) and eleven lizards of *Z. vivipara* from Leningrad (4 females and 1 male) and



Fig. 1. Metaphases of blood cells of females *Zootoca vivipara*. $2n = 35$ (population No. 1). **A**, C-banded karyotype with submetacentric (SV) W_3 -sex chromosome showing three (one centromeric and two telomeric) heterochromatic C-bands, “western” form of *Zootoca vivipara vivipara*; **B**, Chromomycin A_3 stained karyotype showing weak telomeric C-bands of NORs of two chromosomes; **C**, DAPI stained karyotype, showing centromeric and one telomeric C-bands of W_3 -sex chromosome and centromeric C-bands of some autosomes of *Zootoca v. vivipara*.

Pskov (5 females and 1 male) regions, Russia were collected. For clarifying mode of reproduction some females were kept in terrarium up to hatching of offspring.

C-banding was carried out according to Sumner’s method (Sumner, 1972), fluorochrome staining (chromomycin A_3 and DAPI according to Schmid and Guttenbach method (Schmid and Guttenbach, 1988); digestions with endonucleases Alu1 (Mezzanotte et al., 1983).

RESULTS AND DISCUSSION

Males of *Z. vivipara* from these populations had 36 acrocentric chromosomes.

Observations in a terrarium have shown that all specimens belong to advanced viviparous forms.

Karyotype Structure and Identification of Populations

Females of *Z. vivipara* from the Carpathian region (population No. 1) had 35 chromosomes with Z Z W system of sex chromosomes and biarmed meta (V)/submetacentric (SV) W_3 -sex chromosome.

Most of autosomes and W_3 chromosome possessed conspicuous centromeric C-bands. Autosomes had thin telomeric C-bands whereas W_3 chromosome displayed two intensive telomeric C-bands (Fig. 1A).

Telomeric C-bands of the NORs bearing chromosomes were weakly stained with GC-specific fluorochrome chromomycin A_3 (Fig. 1B). Centromeric and one telomeric C-bands of the sex chromosome were weakly stained with AT specific fluorochrome DAPI (Fig. 1C). After treatment with endonuclease Alu1, only the centro-

meric bands of the autosomes and a single band of W_3 chromosome persisted.

From these chromosome markers the specimens of *Z. vivipara* from population No. 1 appeared to be similar to those of specimens belonging to the “western” form of *Z. v. vivipara* from the Trento Alps (Odierna et al., 1998). Therefore examined viviparous lizards from the Carpathian region belong to the “western” form of subspecies *Z. v. vivipara*.

The karyotype of females of *Z. vivipara* from northwestern region of Russia (populations Nos. 2 and 3) had $2n = 35A$, with Z Z W system of sex chromosomes. W sex chromosome was uniarmed acrocentric(A)/subtelocentric (ST) W_2 . Most of autosomes and W_2 chromosome possessed conspicuous centromeric and telomeric C-bands. Additionally W_2 sex chromosome has interstitial C-band (Fig. 2A). From these chromosome markers these specimens of *Z. vivipara* from populations Nos. 2 and 3 belong to the “Russian/eastern” form of *Z. v. vivipara*.

It follows that the cytogenetical data are good markers for identification of populations of *Z. vivipara* throughout the distribution range.

Telomeric C-bands of the NORs bearing chromosomes were intensively stained with GC-specific fluorochrome chromomycin A_3 (Fig. 2B). Centromeric C-bands of some autosomes, centromeric and interstitial C-bands of the W_2 sex chromosome were intensively stained with AT specific fluorochrome DAPI (Fig. 2C). Unlike W_3 -sex chromosome of “western” form after treatment with endonuclease Alu1, the centromeric and interstitial bands of W_2 chromosome were resistant.

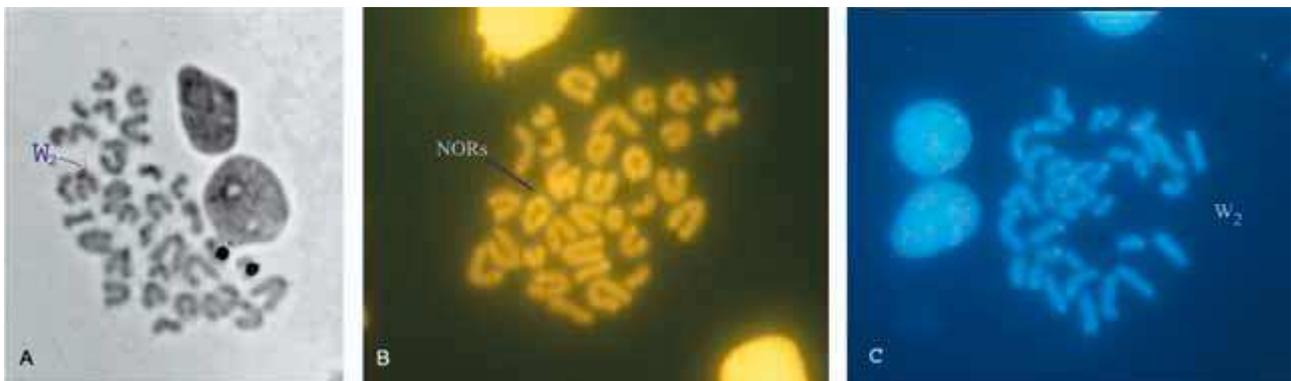


Fig. 2. Metaphases of blood cells of females *Zootoca vivipara*. $2n = 35$ (populations Nos. 2 and 3). **A**, C-banded karyotype with acrocentric (A) W_2 -sex chromosome showing three (centromeric, telomeric and interstitial) heterochromatic C-bands (“Russian/eastern” form of *Zootoca vivipara vivipara*); **B**, Chromomycin A_3 stained karyotype showing intensive telomeric C-bands of NOR regions; **C**, DAPI stained karyotype showing centromeric and interstitial C-bands of W_2 .

Chromosomal Reorganization in the Evolution of *Z. vivipara*

Comparative analyses have revealed that two cytogenetic characteristics of the “Russian/eastern” form are the same as those found by Odierna and his coauthors (Odierna et al., 1998) in the “pyrenean” form. They are: 1. intensively stained with GC-specific fluorochrome chromomycin A_3 telomeric C-bands of the NORs bearing chromosomes; 2. intensively stained with AT specific fluorochrome DAPI centromeric C-bands of some autosomes, centromeric and interstitial C-bands of the W_2 -sex chromosome.

By contrast, specimens of the “western” form displayed other cytogenetical markers.

1. Weakly stained with chromomycin A_3 telomeric C-bands of the NORs bearing chromosomes.

2. Weakly stained with DAPI centromeric and telomeric C-bands of W_3 chromosome.

Thus, cytogenetical data obtained again argue that intensive karyotype reorganization accompany active form-formation and subspeciation in the evolution of *Z. vivipara*. As has been mentioned above, the karyotypes of both primitive “Pyrenean” form and subspecies *Z. v. carniolica* are characterized by low amount of heterochromatine and by narrow range. In contrast, both advanced the “Russian/eastern” and “western” forms of *Z. v. vivipara* with wide range possess a considerable amount of heterochromatine in their karyotype. These data suggest that the latter karyotype is evolutionarily plastic (Kupriyanova and Odierna, 2002). Cytogenetical results obtained seem to be inconsistent with the hypothesis (Heulin et al., 1993) for arising of advanced viviparous form in some region of eastern Europe because primitive oviparous forms have

been observed neither in this region nor in south-eastern populations of Russia yet. Advanced viviparous “Russian/eastern” form of *Z. v. vivipara* inhabits this region (Kupriyanova et al., 2003), whereas “western” form of *Z. v. vivipara* lives in central and western Europe. However “western” form has recently been karyologically found in north-western region of Russia. Both these forms and oviparous *Z. v. carniolica* differing in karyotype structure live in central Europe.

Our data again confirm the assumption (Kupriyanova and Böhme, 1997; Surget-Groba et al., 2001) that the Carpathian basin may be considered as a center of form-formation and subspeciation of *Z. vivipara*. The Baltic basin is a zone of a secondary contact of two forms (Kupriyanova, 1997, 2004). Karyologically *Z. vivipara* constitutes a mosaic of populations inhabiting different European and Asian countries. Conservation of some of these populations is needed (Odierna et al., 2004; Kupriyanova, 2004).

Chromosomes and Modes of Form-Formation and Subspection

In connection with the facts established a question of possible modes form-formation and subspeciation in the evolution of this wideranged Euroasian species arises.

These several morphologically no diagnostic criptic forms of *Z. v. vivipara* have some serious karyotype’s and to a lesser extent haplotype’s differences.

The model of allopatric differentiation is associated with climatic changes. The Pleistocene glaciation could have caused the separation of the original population into two (or more) groups. All populations examined are allopatric or parapatric. No sympatry, hybrid or contact zones have been found.

Modern cytogenetical data show that rearrangement of chromosomes may represent a powerful mechanism for reproductive isolation. For instance, karyological variations in the complex *Lacerta kulzeri* support the King's model of chromosomal primary allopatry (in den Bosch et al., 2003).

Alterations in morphology and/or heterochromatin content of sex chromosomes are known to have a negative impact on hybrid fertility in some rodents (Lyapunova et al., 1990).

We found two the same molecular markers of chromosomes of the primitive "Pyrenean" form and of the advanced "Russian/eastern" form of *Z. v. vivipara*. Interestingly, the shape and heterochromatin distribution of W chromosome of these two forms are very similar (Odierna et al., 1998, 2001) and furthermore the adaptive value of viviparity has been showed in this lizard (Odierna et al., 2004). These data obtained allow us to consider another scenario. They may suggest that chromosomal reorganization could have accompanied colonization and adaptive radiation events.

The next tasks to be investigated are:

Summarizing we would like to emphasize that now we have different information about *Z. vivipara* complex but it is still not enough for understanding the situation. Further international cytogenetical researches of this wide-ranged Euroasian species should attempt to clarify and to test several aspects of the process of form-formation and subspeciation and biogeography in order to

1. karyologically to identify a larger number of populations to determine the quantity of different forms and subspecies and to clarify their distribution range;
2. to protect or to conserve some rare populations or those of them with narrow range;
3. to find new molecular markers of chromosomes of these cryptic forms and subspecies using modern techniques;
4. to precise *in situ* localization of specific (sex linked) genes;
5. to resolve the questions about taxonomic status and phylogenetic relationships of discovered chromosomal forms of *Z. v. vivipara*.

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