

Evolutionary and biogeographical implications of the karyological variations in the oviparous and viviparous forms of the lizard *Lacerta (Zootoca) vivipara*

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The lizard *Lacerta (Zootoca) vivipara* has two modes of reproduction and is variable karyologically. We describe its karyological variation from literature data and from new data on two viviparous populations from France, on two oviparous populations from the Pyrénées in south-western France and on three oviparous populations recently discovered in Slovenia. Males have 36 chromosomes, whereas females have only 35 chromosomes in all viviparous populations and in the Pyrenean oviparous populations. This karyotype has been interpreted to result from a fusion of an ancestral sexual W chromosome with an autosome from the Z1 or from the Z2 pair. The karyotype formula is 32 autosomes + Z1Z2W for the female and 32 autosomes + Z1Z1Z2Z2 for the male. The karyotype of the Slovenian oviparous populations, 34 autosomes + ZZ in the male and 34 autosomes + ZW in the female, represents an evolutionary stage that preceded the chromosomal fusion. There is minor karyological variation, mainly concerning the W and Z2 chromosomes, within the Pyrenean oviparous populations. This parallels the geographic variation of the W-linked alleles of the MPI enzyme and suggests that allopatric differentiation of these oviparous populations might have occurred in the vicinity of the Pyrénées during the Pleistocene.

The viviparous populations from western Europe carry a metacentric W chromosome, whereas oviparous populations from south-western Europe and eastern viviparous populations both show an acrocentric, or a subtelocentric, W chromosome. This suggests that the acrocentric-subtelocentric W is a primitive character and that viviparity probably arose in an eastern lineage of the species.

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The lizard *Lacerta (Zootoca) vivipara* has two reproductive modes and is variable karyologically. Together with its extensive geographic range, it is an ideal model in biogeographic and evolutionary studies.

Lacerta (Zootoca) vivipara is the only Lacertid that has both oviparous and viviparous populations. The

populations from central France and the British Isles up to Scandinavia and eastern Russia, are viviparous. Oviparous populations were first identified in the extreme south-western part of the species' range, from the Cantabric mountains in Spain to the Pyrénées and Aquitaine regions in France (Lantz 1927, Brana and

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Bea 1987, Heulin 1988, Heulin and Guillaume 1989). No contact zones have been found between oviparous and viviparous populations in southern France (Heulin and Guillaume 1989). Characteristics of viviparity (with persistence of non-functional oviparous structures), morphological and ecological resemblances, successful experimental (laboratory) hybridizations and small genetic distances calculated from allozymes and mtDNA studies all indicate that oviparous and viviparous strains of *L. (Z.) vivipara* are very closely related and are likely to have diverged recently (Heulin 1990, Bea et al. 1990, Heulin et al. 1992, 1993, 1999, Arrayago et al. 1996, Guillaume et al. 1997). Pleistocene events have been suggested to account for this recent evolution and the geographic distribution of the two reproductive forms (Heulin et al. 1993). The original populations (presumably still oviparous) could have been forced to retreat to the southwest and southeast during glacial periods. Viviparity could have evolved among the south-eastern populations, somewhere between the Balkan Peninsula and the south of Russia, whereas the south-western populations remained oviparous in the Pyreneo-iberian region. During interglacial periods (including the present), the viviparous populations would have (re)colonized north-eastern and north-western regions, without any secondary contact with the residual oviparous populations in the south-western (Pyreneo-iberian) area (Heulin et al. 1993). The time scale of this scenario agrees with our allozyme and mtDNA analyses, which both suggest that the differentiation of the two reproductive forms of *L. (Z.) vivipara* could have begun between -2MY and -0.5MY b.p. (Heulin et al. 1999). Moreover, geographic variation of the shape of the W chromosome (Odierna et al. 1998, and see below) seems to support our hypothesis that the center of origin of viviparity of *L. (Z.) vivipara* should be located in an eastern region. Until recently, oviparous populations have not been found in this area. The recent discovery of oviparous populations of *L. (Z.) vivipara* in Slovenia (Böhme et al. 1999, Heulin et al. 2000) raises the question whether these Slovenian oviparous populations could have given rise to viviparity.

Lacerta (Zootoca) vivipara has an unusual karyotype. Males have 36 chromosomes, while females only have 35 chromosomes. This particular karyotype has been observed in numerous viviparous populations and in two Pyrenean oviparous populations (Chevalier et al. 1979, Kupriyanova 1986, 1990, Belcheva et al. 1986, Kupriyanova and Rudi 1990, Odierna et al. 1993, 1998, Kupriyanova et al. 1995, Kupriyanova and Böhme 1997, Orlova and Kozlovskii 1997). It has been interpreted as a sex-chromosome system in which two chromosomes from distinct pairs have undergone a fusion in the female. The karyotype formula of the male is 32 autosomes + Z1Z1 + Z2Z2 and the karyotype formula of the female is 32 autosomes + Z1 + Z2 + W. How-

ever, we do not know whether the primitive karyotype (females with 36 chromosomes) still persists somewhere.

Variation in the shape of the female sexual chromosome has also been documented (Fig. 1). A biarmed (metacentric) W chromosome characterizes the populations from the western part of the distribution area of the viviparous form (Chevalier 1969, Chevalier et al. 1979, Belcheva et al. 1986, Odierna et al. 1993, Kupriyanova et al. 1995), whereas an unarmed (acrocentric) or nearly unarmed (subtelocentric) W has been observed both in the eastern part of the distribution range of the viviparous form (Oguma 1934, Kupriyanova 1990, Kupriyanova and Rudi 1990, Kupriyanova et al. 1995, Kupriyanova and Böhme 1997, Orlova and Kozlovskii 1997) and also in the two Pyrenean oviparous populations recently studied by us (Kupriyanova and Böhme 1997, Odierna et al. 1998). Given that oviparity is the ancestral reproductive mode in Reptiles, we may assume that in *L. (Z.) vivipara*, the acrocentric - or subtelocentric - form of the W corresponds to an ancestral character, whereas the metacentric form of the W corresponds to a derived character. We may deduce that 1) an oviparous lineage carrying an acrocentric-subtelocentric W would have produced a viviparous lineage still carrying an acrocentric-subtelocentric W and 2) that a pericentric inversion occurring in this first viviparous group would have subsequently given rise to another viviparous lineage carrying a metacentric W. If so, one can predict that all the oviparous females of *L. (Z.) vivipara* should necessarily have an acrocentric-subtelocentric W. We shall test this prediction by examining the karyological characteristics in several oviparous populations and, in particular, the ones recently discovered in Slovenia.

We will extend our data base by examining the karyological characteristics of two viviparous populations from France, of two Pyrenean oviparous populations, and of three oviparous populations recently discovered in Slovenia. We will discuss to what extent these new data throw light on the evolutionary and biogeographical problems mentioned above.

Material and methods

We studied the karyological characteristics of 25 specimens from different geographic regions. The Slovenian oviparous sample was: 1 male and 1 female from Kot in the Pohorje Mounts (46°26'N, 15°26'E), 1 male and 1 female from Zelenci (46°30'N, 13°44'E) and 1 male and 1 female from the banks of Cerknisko Lake (45°46'N, 14°22'E). The French oviparous sample from western Pyrénées was: 2 males and 4 females from Louvie (43°06'N, 0°3'E) on the north of Vallée d'Ossau, and 3 males and 3 females from the Pourtalet (42°50'N, 0°3'E) on the south of Vallée d'Ossau (i.e. France-Spain bor-

der). These two Pyrenean oviparous populations are known to differ in their sex-linked alleles of the MPI enzyme (Mannose Phosphate Isomerase). Females from Louvie carry the fast migrating allele MPI¹¹⁰, whereas females from Pourtalet carry the slow migrating allele MPI⁹⁰ (Bea et al. 1990, Guillaume et al. 2000). The French viviparous sample was 2 males and 2 females from Paimpont (48°N, 2°W) in Bretagne and 1 male and 2 females from Chambéry (45°32'N, 6°E) in the Alps. The localization map of these populations and of the other European populations, whose karyological characteristics have previously been studied, is presented in Fig. 1.

Preparation of karyological plates, staining of the nucleolar organizer region (NOR) and C-banding staining of the chromosomes were performed according to the procedures previously presented in Odierna et al. (1993, 1998).

Our identification of different karyotypes was primarily based on the characteristics, i.e., number of chromosomes, sex-chromosome system, shape of the W chromosome, NOR position, which were previously described for numerous viviparous populations (Chevalier et al. 1979, Belcheva et al. 1986, Kupriyanova 1986, 1990, Kupriyanova and Rudi 1990, Odierna et al. 1993, 1998, Kupriyanova et al. 1995, Kupriyanova and Böhme 1997, Orlova and Kozlovskii 1997) and for two oviparous populations (Kupriyanova and Böhme 1997, Odierna et al. 1998). However, we also attempted to take into account the more detailed information on the heterochromatin distribution on the chromosomes, which has been obtained by banding analysis for some of these populations. Literature data including description of C-banding staining were only available for an oviparous population from central Pyrénées and for the viviparous populations from Italian Alps, from the

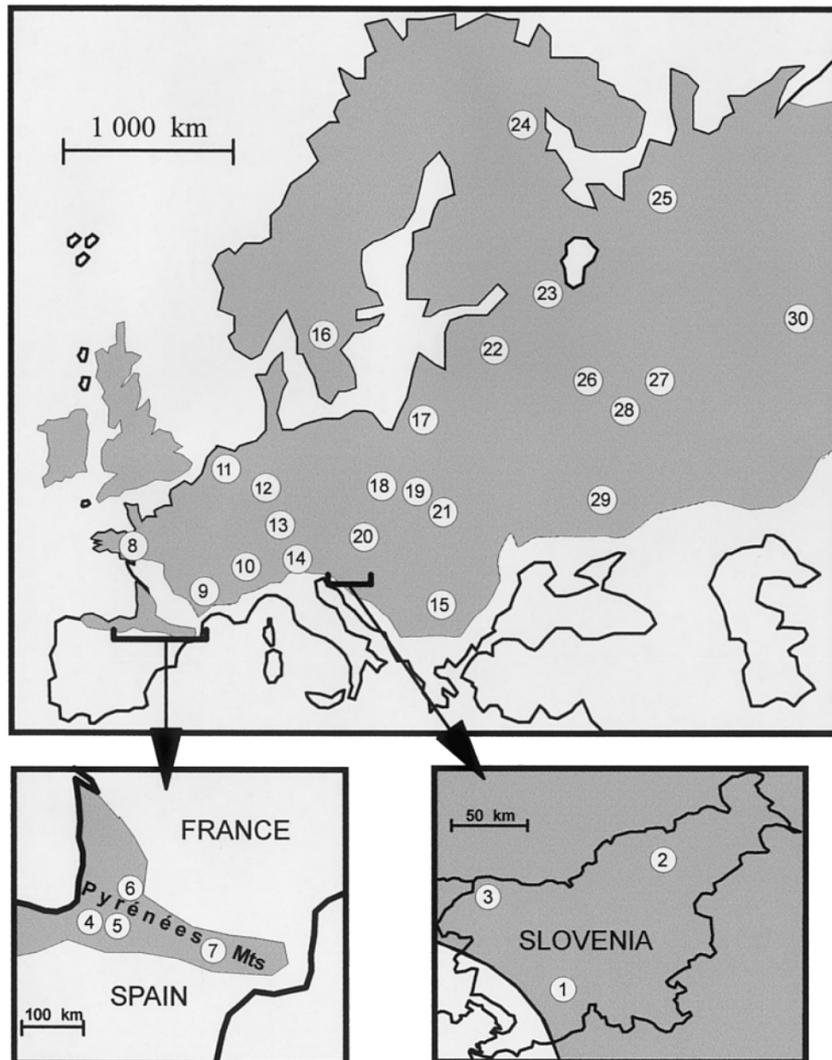
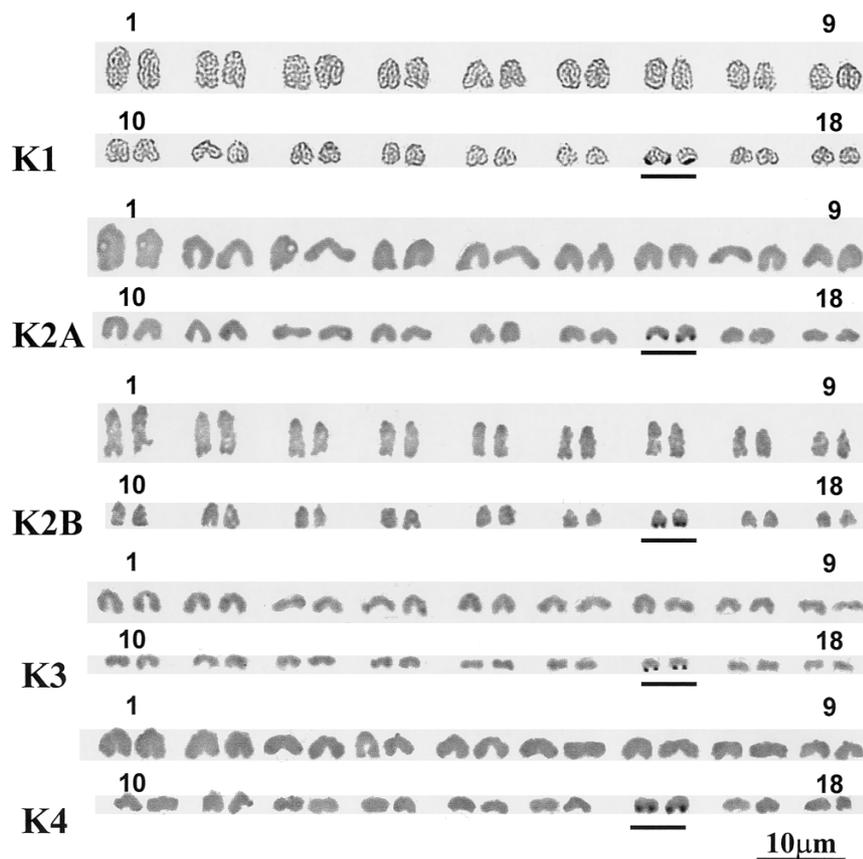


Fig. 1. Localization map of the European populations of *L. (Z.) vivipara* whose karyotype formulas are known. 1–3: Slovenian oviparous populations. 4–7: Pyrenean Oviparous populations. 8–19: Western viviparous populations (with metacentric W: see text). 20–30: eastern viviparous populations (with acrocentric-subtelocentric W: see text). Data from this study: Cerknisko lake 1, Kot-Pohorje 2, Zelenci 3, Pourtalet 5, Louvie 6, Paimpont 8, Chambéry 10. Literature data: from Belcheva et al. (1986) for Bulgaria 15; from Chevalier (1969) for Massif central 9; from Kupriyanova (1990) for western Carpathes 18, Shirokii lug region in eastern Carpathes 19, Chernogorya region in eastern Carpathes 21 and Oural 30; from Kupriyanova et al. (1995) for Sweden 16, Kaliningrad region 17 and Karelia 24; from Kupriyanova and Böhme (1997) for south-western Spanish Pyrénées 4, Germany 12, Switzerland 13, eastern Austria 20, St. Petersburg region 23, Arkhangelsk region 25 and Karkhov region 29; from Odierna et al. (1993) for the Netherlands 11, and Pskov region 22; from Odierna et al. (1998) for central Pyrénées 7 and Italian Alps 14; from Orlova and Kozlovskii (1997) for Tver region 26, Ivanovo region 27 and Moscow region 28.

Fig. 2. Ag-NOR banded karyotypes of males of *L. (Z.) vivipara* from different origins. The pair of chromosomes carrying the nucleolar organizer region (NOR) is underlined. K1 Slovenian oviparous specimen from Zelenci (this study); K2A oviparous specimen from Louvie in north-western Pyrénées (this study); K2B oviparous specimen from Pourtalet in south-western Pyrénées (this study); K3 viviparous specimen from Pskov in Russia (Odierna et al. 1993); K4 viviparous specimen from Paimpont in France (this study).



Netherlands and from Pskov region in Russia (Odierna et al. 1993, 1998).

Results

Oviparous populations from Slovenia

We did not observe karyological differences between the three Slovenian oviparous populations studied. Males and females have the same number ($2N = 36$) of chromosomes and the NOR (nucleolar organizer region) is localized on the telomere of a medium-small pair of chromosomes (Figs 2 and 3; diagrammatic representation of the female's karyotype: K1 in Fig. 4). The males have 36 acrocentric macro-chromosomes, whereas the females have 35 acrocentric macro-chromosomes + 1 micro-chromosome. This indicates that the Slovenian populations have a karyotype with a sex-chromosome system of ZW type. The male has two sexual macro-chromosomes Z and the female has 1 sexual macrochromosome Z and 1 sexual micro-chromosome W.

Weak centromeric C-bands of heterochromatin are observed on some autosomes and on the Z chromosome. The W micro-chromosome is almost fully heterochromatic.

Oviparous populations from the Pyrénées

Males of the two Pyrenean oviparous populations studied, have $2N = 36$ macro-chromosomes and the females have only $2N = 35$ macro-chromosomes (Figs 2 and 3; diagrammatic representation of the female's karyotype: K2A and K2B in Fig. 4). As explained above, this particular formula can be interpreted as corresponding to the existence of a $Z1Z2W$ sex-chromosome system. The male karyotype is 32 autosomes + $Z1Z1 + Z2Z2$ and the female karyotype is 32 autosomes + $Z1Z2W$. The autosomes and the Z1 chromosome are acrocentric in the two populations. The chromosomes W and Z2, which are also acrocentric in Louvie, have, however, a second very short arm (i.e. subtelocentric chromosomes) in the Pourtalet population.

In the two populations the NOR is localized on the telomere of a medium-small chromosome (Fig. 2). Pyrenean oviparous lizards possess autosomes which are only weakly heterochromatinized at the centromere. The pattern of heterochromatin distribution on the autosomes does not differ strongly between Louvie (K2A) and Pourtalet (K2B). In contrast, there is an obvious difference of heterochromatin distribution on the W chromosome between the two populations. The W chromosome has a large interstitial band of hete-

rochromatin in the Pourtalet population, whereas this feature does not exist in the Louvie population (see Karyotype K2B/karyotype K2A: photos in Fig. 3, diagrams in Fig. 4).

French viviparous populations

We did not observe karyological differences between the two French (Paimpont, Chambéry) viviparous populations studied. In these viviparous populations, males have $2N = 36$ macro-chromosomes and females $2N = 35$ macro-chromosomes (Figs 2 and 3; diagrammatic representation of the female's karyotype: K4 in Fig. 4). As for the Pyrenean oviparous populations, the karyotype of these French viviparous populations can be interpreted as 32 autosomes + $Z1Z1 + Z2Z2$ for the male and 32 autosomes + $Z1Z2W$ for the female. The NOR is also localized on the telomere of a medium-small chromosome. The W chromosome of females has two long arms (i.e. metacentric), whereas other chromo-

somes of males and females are acrocentric. The W chromosome has a conspicuous paracentromeric band and two telomeric bands of heterochromatin. The chromosomes Z1, Z2 and all autosomes have intense centromeric bands. The chromosome Z1 has a thin interstitial band. Telomeric bands were observed in all the autosomes.

Discussion

Comparison with literature data

The present study allowed us to distinguish the karyotypes K1, K2A, K2B and K4 of *L. (Z.) vivipara*. According to Odierna et al. (1993, 1998) it is also possible to recognize a fifth karyotype (called K3 in our Figs. 2–4).

The karyotype K1 (females with $2N = 36$ and ZW system) has not been reported previously. Previous reports of $2N = 36$ chromosomes both in males and in

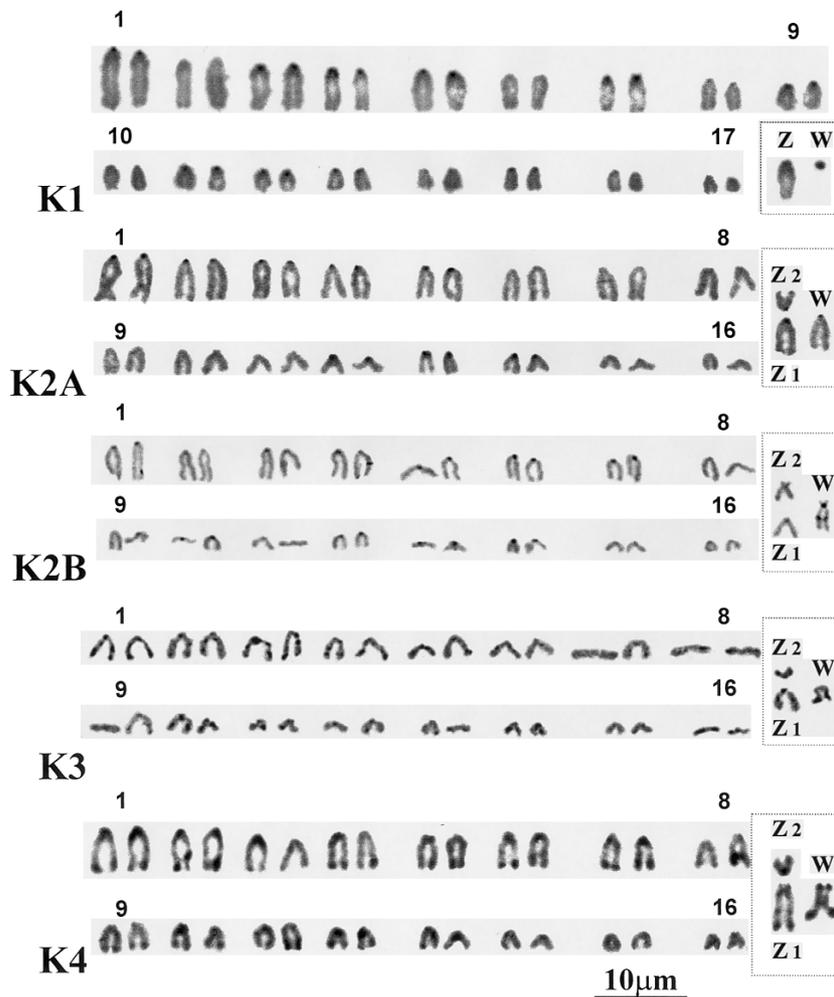


Fig. 3. C-banded karyotypes of females of *L. (Z.) vivipara* from different origins. Origins of the specimens as in Fig. 2. The autosomes and the sex chromosomes (in the squares) are distinguished. The females' karyotype formula is 34 autosomes + $Z + W$ in the oviparous populations of Slovenia; whereas it is 32 autosomes + $Z1 + Z2 + W$ in all other populations (see text).

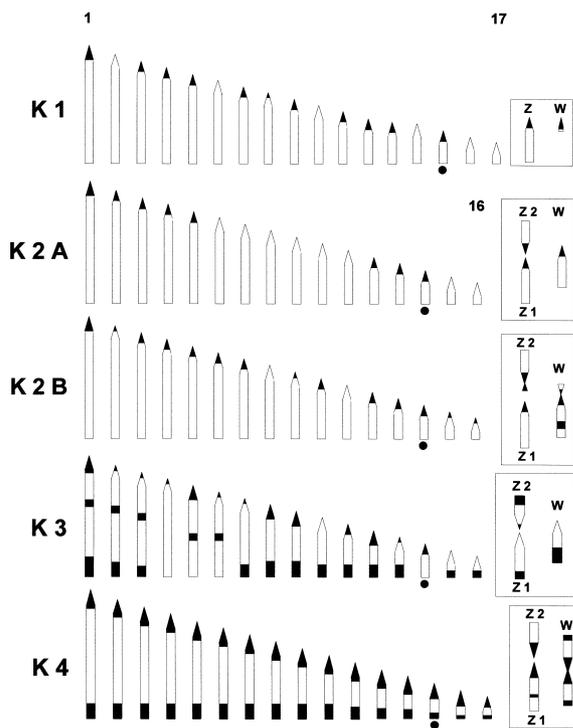


Fig. 4. Diagrammatic representation of the C-banded chromosomes in females of *L. (Z.) vivipara* from different origins. The black dot indicates the localization of the NOR. K1 of the Slovenian oviparous populations (this study); K2A in the oviparous populations from Louvie in north-western Pyrénées (this study) and from Viella in central Pyrénées (Odierna et al. 1998); K2B in the oviparous population from Pourtalet in south-western Pyrénées (this study); K3 in the viviparous population from Pskov in Russia (Odierna et al. 1993); K4 in the viviparous populations from Paimpont and Chambéry in France (this study), and from Italian Alps and the Netherlands (Odierna et al. 1998).

females, in the Moscow region (Orlova and Orlov 1969) and in Switzerland (Margot 1946, Matthey and Van Brink 1956), are probably wrong and due to old cytological techniques (Odierna et al. 1998). A karyological re-investigation of populations from the Moscow region and from Switzerland shows that females from these populations have 35 chromosomes and the typical Z1Z2W system observed elsewhere (Kupriyanova and Böhme 1997, Orlova and Kozlovskii 1997, cf. also the unpublished data of Matthey cited in Chevalier et al. 1979).

We previously documented the existence of a Z1Z2W system in two Pyrenean oviparous populations. As in Pourtalet (karyotype K2B), the W chromosome was subtelocentric (i.e. with a second very short arm) in a Spanish oviparous population located on the southwest of the Pyrenean range (Kupriyanova and Böhme 1997, population 4 in Fig. 1) whereas, as in Louvie (karyotype K2A), the W chromosome was acrocentric in an oviparous population from central Pyrénées (Odierna et

al. 1998, population 7 in Fig. 1). The population of central Pyrénées studied by Odierna et al. (1998) had a distribution pattern of heterochromatin identical to that in Louvie.

The karyotype K4 (i.e. Z1Z2W system with a biarmed metacentric W) is a characteristic of the western viviparous populations of the species: it has been observed in the viviparous populations studied in central France, the Netherlands, Italian Alps, Bulgaria, Sweden and Baltic regions (Chevalier 1969, Chevalier et al. 1979, Belcheva et al. 1986, Odierna et al. 1993, 1998, Kupriyanova et al. 1995) (see populations 8–19 in Fig. 1). Investigations including C-banding staining were performed for the population from the Netherlands and for the population from Italian Alps (Odierna et al. 1998). In both cases, the distribution pattern of heterochromatin bands on the chromosomes was identical to those observed in the French populations of Chambéry and Paimpont.

The karyotype K3 (i.e. Z1Z2W system with an acrocentric or subtelocentric W) is a characteristic of the eastern viviparous populations, from north-eastern Austria, Ukrainian Carpathes and European Russia up to northern Kazakhstan and Sakhalin Island (Oguma 1934, Kupriyanova 1986, 1990, Kupriyanova and Rudi 1990, Kupriyanova et al. 1995, Kupriyanova and Böhme 1997) (see European populations 20–30 in Fig. 1). It is not yet clear whether the acrocentric and subtelocentric forms of the W chromosome have different geographic distributions among eastern viviparous populations, although this chromosome has been described as being subtelocentric in central European Russia (populations 26–28 in Fig. 1) and as being generally acrocentric in other populations of the eastern group. There is, however, evidence of instability in the shape of the W chromosome within the eastern viviparous group. In particular, a mosaic pattern, that is a variation of the shape (acrocentric/subtelocentric) of the W chromosome within a single individual, has been reported (Kupriyanova 1990, Kupriyanova and Rudi 1990, Kupriyanova and Böhme 1997). The C-banding staining has only been applied to a single population of the eastern viviparous group (Odierna et al. 1993). This technique revealed the existence of intense telomeric bands on the W, Z1 and Z2 chromosomes and of numerous bands, in various positions, on most of the autosomes (see K3 in Figs 3 and 4).

The two karyotypes K3 and K4, identified in the viviparous populations, have distinct distributions of heterochromatin bands, but have, nevertheless, similar total amounts of heterochromatin. In contrast, it must be noted that the chromosomes of the viviparous forms are much more heterochromatinized than those of the oviparous forms. Therefore, the evolution of viviparity in *L. (Z.) vivipara* has certainly been followed by, or is associated with, an intense heterochromatinization.

Evolutionary and phylogeographic inferences

We shall attempt to identify the phylogeographical relationships among the different karyotypes of *L. (Z.) vivipara*.

Most Lacertid species have chromosome numbers (from $2N = 24$ to $2N = 42$) that are identical in males and females (Chevalier et al. 1979, Olmo et al. 1987, 1991, Volubiev et al. 1990, Odierna et al. 1993, 1996). In these Lacertids, the sex chromosomes are either homomorphic or of ZW type. The differentiation of the sex chromosomes seems to have occurred repeatedly in Lacertid lineages through a gradual process of heterochromatinization and of shortening (due to the deletion of some heterochromatinized area) of the W (female) chromosome. The primitive stage is found in species with Z and W chromosomes of the same size, poorly heterochromatinized and almost homomorphic, and a derived stage is found in species with a strongly heterochromatinized microchromosome W, smaller than the Z chromosome (review in Odierna et al. 1993). Independently of this general evolutionary trend in the Lacertidae, a particular cytogenetical event occurred in *L. (Z.) vivipara*. The unequal number of chromosomes in males and females (respectively 36 and 35 chromosomes) has been interpreted to result from a fusion of an ancestral W chromosome with an autosome, giving rise to a neo-W and to a particular sex-chromosome system called Z1Z2W (see Introduction). Recent research showed that a similar cytogenetical event also occurred in the Iberian rock lizard group in which *Lacerta aurelioi* and *Lacerta bonnali* actually have an unequal number of chromosomes in males and females and a Z1Z2W heterogametic system (Odierna et al. 1996). *Lacerta (Zootoca) vivipara* is not phylogenetically closely related to the Iberian rock lizard group (Arnold 1989, Mayer and Benyr 1994, Harris et al. 1998). Therefore, the evolution of the Z1Z2W system of *L. (Z.) vivipara* evidently occurred independently of the evolution of the same sex chromosome system in the Iberian rock lizard group.

The karyotypes K2A, K2B, K3 and K4 of *L. (Z.) vivipara* all have the Z1Z2W heterogametic system described above. The most striking result obtained in our study is that the Slovenian oviparous lineage (karyotype K1) has not undergone the chromosomal fusion which subsequently gave rise to the Z1Z2W sex-chromosome system. The Slovenian populations of *L. (Z.) vivipara* have a chromosomal formula postulated for the ancestor (with ZW system) of all other oviparous and viviparous lineages (with Z1Z2W system) of this species. We do not know whether this hypothetical ancestor had a microchromosome W strongly heterochromatinized (as in extant Slovenian populations) or a larger and less differentiated W (see above: progressive differentiation of the W in Lacertidae). Nevertheless, the Slovenian oviparous lineage has females with

$2N = 36$ chromosomes, a circumstance that is clearly more primitive than that observed in other oviparous and viviparous populations. Viviparity can therefore not have directly originated from the Slovenian oviparous group.

The presence of a Z1Z2W system in the Pyrenean oviparous populations (i.e. karyotypes K2A and K2B) indicates that the W + autosome fusion occurred before the evolution of viviparity, in an oviparous lineage. We do not know whether the fusion has given rise to an oviparous lineage with a karyotype similar to K2A or to K2B. As the evolution of the W chromosome is often associated with a heterochromatinization of this chromosome in the Lacertidae, the presence of an additional interstitial band of heterochromatin on the W chromosome of the karyotype K2B, might indicate that this karyotype K2B appeared later than, and possibly derived from, the karyotype K2A. The evolutionary transition from K2A to K2B, would also have implied a small displacement of the centromere from an acrocentric position to a sub-telocentric position, both in the W chromosome and in the Z2 chromosome. Overall, the slight autosomal differentiation and the close geographic proximity of the karyotypes K2A and K2B suggest that these two karyotypes are likely to be phylogenetically closely related. Nevertheless, the karyotypes K2A and K2B may have distinct geographical distributions. The former was observed in the north-western and central Pyrénées, whereas the latter was observed in the south-western part of the Pyrénées. This geographic variation of karyotypes among Pyrenean oviparous populations, parallels those of the W-linked alleles of the MPI (Mannose Phosphate Isomerase) enzyme. Females from the eastern, central and north-western Pyrénées (including Louvie) are monomorphic for the fast migrating MPI alleles, whereas females from the Cantabric mountains and the south-western Pyrénées (including Pourtalet) are monomorphic for the slow migrating MPI allele (Bea et al. 1990, Guillaume et al. 2000). This might reflect an allopatric differentiation of two oviparous sub-groups that retreated to distinct refuges in the Pyreneo-iberian region during the Pleistocene (Guillaume et al. 2000).

The eastern viviparous populations (karyotype K3) and the Pyrenean oviparous populations (karyotypes K2A and K2B) both have an acrocentric or subtelocentric W, which is different from the metacentric W observed in the western viviparous populations (Karyotype K4). This suggests that viviparity first arose in an eastern lineage still carrying an acrocentric-subtelocentric W, and that the metacentric W represents a derived character which arose within the viviparous group. Kupriyanova and Rudi (1990) and Odierna et al. (1998) hypothesized that a pericentric inversion, giving rise to a W with two long arms, would have only occurred in the viviparous lineage that colonized western Europe.

The major phylogeographic problem arising from the above interpretation is that oviparous populations with the expected characteristics (i.e. females with 35 chromosomes, Z1Z2W system and acrocentric or subtelocentric W) have not yet been found in the vicinity of the eastern viviparous group. The fact that the extant oviparous populations are restricted to regions (NW Spain, SW France, Slovenia) of relatively low latitude as compared to those occupied by most of the viviparous populations of this species agrees with the adaptive predictions of the “cold climate” theory (see Shine 1985, Heulin et al. 1991, 1997). Accordingly, we predict that our hypothetical eastern oviparous populations with acrocentric-subtelocentric W would likely be localized somewhere between the south of central Europe and the south of Russia.

Conclusion – prospects

We still need more data, on characters such as karyotypes and mt DNA, to test the validity of our phylogeographic interpretations. A point that we wish to emphasize is that alternative scenarios seem to be more complex and less plausible. For example, the hypothesis of two independent origins of viviparity (one occurring within an eastern lineage with acrocentric-subtelocentric W and the other within a western lineage with metacentric biarmed W) is unlikely, because our recent mtDNA analyses indicate that the viviparous populations of *L. (Z.) vivipara* form a monophyletic group (Heulin et al. 1999, Surget-Groba unpubl.) and because neither of the oviparous populations as yet studied has a metacentric biarmed W. Similarly, the possibility of two successive pericentric inversions, the first one occurring simultaneously with the evolution of viviparity and giving rise to viviparous lizards with biarmed W, and the second (reverse) one leading subsequently to viviparous lizards with uniarmed W, is also a less likely scenario.

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