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CHROMOSOMAL EVIDENCE FOR THE DOUBLE ORIGIN OF VIVIPARITY IN THE EUROPEAN COMMON LIZARD, *LACERTA (ZOOTOCA) VIVIPARA*

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The results of a chromosomal analysis conducted in a relict, viviparous, Hungarian (Ócsa) population of *L. vivipara*, show that viviparity independently arose in this population. Chromosome characteristics of Ócsa, namely females displaying 2n=36 chromosomes with a ZW sex chromosome system and W shaped as microchromosome, are peculiar to *carniolica*, an early diversifying clade including oviparous population, and different both from oviparous Pyrenean + Aquitainean populations and viviparous Centroeuropean + Eastern populations, whose females possess 2n=35 chromosomes with a Z_1Z_2W sex chromosome system, and W shaped as macrochromosomes.

Key words: evolution, lacertid, reptile viviparity, sex chromosomes

The European common lizard, *Lacerta* (*Zootoca*) *vivipara*, is unique among the lizards in reaching and crossing the Arctic polar circle, and the only lacertid species to display reproductive bimodality, so far only found in two other species of squamates. In fact, *Z. vivipara* possesses both widely distributed, long recognised, viviparous populations (from Central France and the British Isles up to Scandinavia and eastern Russia, until to Sakhalin Island in the Japanese Sea) and two more recently discovered groups of oviparous populations. A first group of allopatric, oviparous populations is restricted to the Pyrenees and Aquitaine in France (Braña & Bea, 1987), and the second group of oviparous populations, assigned to *L. v. carniolica* (Mayer *et al.*, 2000), was found in northern Italy, southern Austria, Slovenia and Croatia (Mayer *et al.* 2000; Surget-Groba *et al.* 2002). Chromosomal analysis (Odierna *et al.* 2001) evidenced that oviparous populations of *carniolica* are karyologically differentiated from all the other populations of *vivipara*: females of *carniolica* display a chromosome complement of 2n=36 chromosomes, a ZW sex chromosome system and W shaped as microchromosome. All the other populations of *vivipara*, including the oviparous populations from the Pyrenees and Aquitaine, instead, display females with a complement of 2n=35 chromosomes, a Z_1Z_2W sex chromosome system, and W shaped as macrochromosomes, whose morphology and heterochromatin distribution have allowed four cytotypes to be detected (Fig. 1; refer also to Odierna *et al.*, 2001, for further details). As shown by mtDNA analysis (Surget-Groba *et al.* 2001), *carniolica* and each of the four cytotypes of *vivipara* display characteristic haplotypes, allowing their identification and relationships (Fig. 1). Karyological and mtDNA results also indicate that the evolution of viviparity was a single event occurring in the clade of central European+eastern viviparous populations (Odierna *et al.*, 2001; Surget-Groba *et al.*, 2001). This evidence is here challenged by the results obtained from a karyological analysis performed on one male and two females of a relict, viviparous (Puky *et al.* 2003), Hungarian population, namely from Ócsa. Chromosomes were obtained by means of the standard air-drying method. Besides conventional staining (5% Giemsa at pH 7), Ag-NOR banding and sequential C-banding+CMA_3+DAPI staining were also performed (Odierna *et al.*, 2001). The studied male and females displayed the same number of 2n=36 chromosomes. As in all the populations studied so far (Odierna *et al.*, 2001), NORs were on the telomeric regions of a small chromosome pair (Fig. 2A). However the male had 36 telocentric macrochromosomes, whereas the females had 34 acrocentric macroautosomes + ZW sex chromosome pair, the W being shaped as a microchromosome while Z was (tentatively) a chromosome as large as those of the sixth pair (Fig 2A). C-banding staining evidenced centromeric and telomeric C-bands on all the autosomes and on the Z chromosome. The W microchromosome was fully heterochromatic and proved DAPI positive after sequential staining of C-banding+CMA_3+DAPI (Fig. 2D). This staining also revealed that centromeric C-bands were weakly DAPI positive (Fig. 2D). Telomeric regions of all autosomes were also weakly CMA_3 positive, except for a small autosome pair, whose telomeric NOR regions were intensely stained. Interestingly, the karyological characteristics of viviparous population of Ócsa are peculiar to the oviparous populations of *Z. v. carniolica*. The ZW sex chromosome system with W shaped as a microchromosome has been found in several other, unrelated, lacertid species and thought as derived from a primitive macrochromosome W, homomorphic to Z, by a multistep process, proceeding formerly by heterochromatinization and successively by progressive events of deletions (Odierna *et al.* 1993; Olmo *et al.* 1993; in den Bosch *et al.* 2003). In our case, this W processing might have occurred either once, consequently the Ócsa population groups within *carniolica* (our preferred hypothesis, since heterochromatin of their W is DAPI positive and Alu I resistant) or twice, then the population of Ócsa constitutes or belongs to a clade

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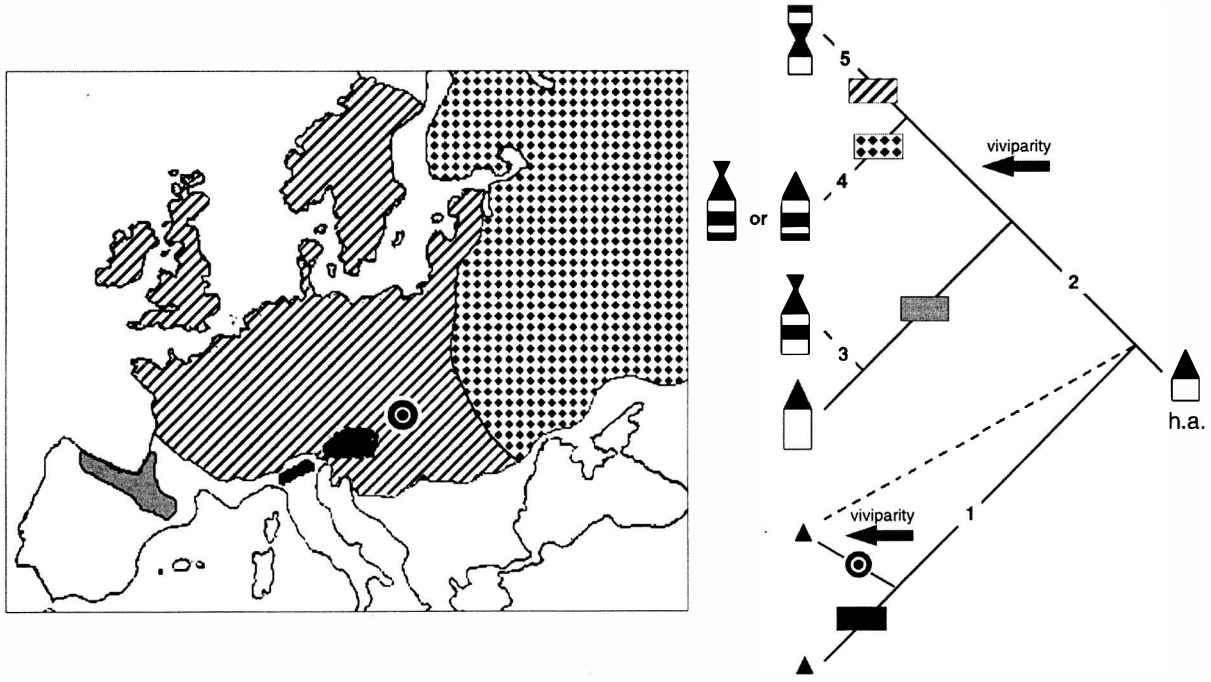

FIG. 1 Gross distribution of the five cytotypes of *L. vivipara* (Asian distribution not included) and the cladogram (modified from Odierna et al., 2001) showing their relationships and characteristics of W chromosome. Eastern viviparous populations ; Centroeuropean viviparous populations ; Pyrenean+Aquitanean oviparous populations ; *L. v. carniolica* oviparous populations ; Ócsa population ; Sex chromosome differentiation, starting from a hypothetic ancestor (h.a.), possessing 2n = 36 acrocentric chromosome, a ZW sex chromosome system, W homomorphic to Z, occurred according to two different modalities: by (1) heterochromatinization of the primitive W and progressive deletion events, shaping the W as microchromosome (the hatched line considers the possibility of an independent origin of the microchromosome W in the Ócsa population); by (2) a tandem fusion of the original W with an autosome to give rise to a Z_1Z_2W sex chromosome system, W shaped as a macrochromosome; afterwards, addition of interstitial (3) and telomeric (4) heterochromatin, as well as a pericentromeric inversion (5) occurred.

distinct from *carniolica* (Fig. 1). However, irrespective of whether the Ócsa population does or does not group within *carniolica*, ours results provide evidence that viviparity independently arose twice, in the population of Ócsa and elsewhere in the clade including Pyrenean+Aquitainean, Centro-European and Eastern populations. Females of these populations possess a Z_1Z_2W sex chromosome system, which is thought derived formerly by a tandem fusion of the primitive W with an autosome and successively by heterochromatinization and/or structural rearrangements (Fig. 1) (see also Odierna *et al.* 1993, 2001; Olmo *et al.* 1993). Multiple sex chromosome systems (Z_1Z_2W or X_1X_2Y) are quite rare in vertebrates, and in lacertids, in addition to *L. vivipara*, have been found only in the Pyrenean populations referred to the *L. bonnali* complex, a group unrelated to *vivipara* and characterised by a number of centric fusions (Odierna *et al.*, 1996). Then, in *L. vivipara* the origin of Z_1Z_2W sex chromosome system constitutes a shared derived character for the Pyrenean-Aquitainean, Centro-European and Eastern populations and splits them off from the other populations with ZW sex chromosome system (*carniolica* and Ócsa). On the other hand, we also exclude that the microchromosome displayed by females of Ócsa should be either a B (supernumerary) chromosome or the result of an event of centric fission occurring in the subtelocentric W displayed by females of Pyrenean-Aquitenean, Centro-European and Eastern populations, or a by product of the introgression of maternal markers from *carniolica* within a viviparous genome. B elements are randomly inherited and we found none of these elements in the Ócsa male, as well as in number of examined specimens of *L. vivipara*. Furthermore, supernumerary elements are quite rare among lacertids, since they have so far been found in only two species (Olmo *et al.*1993). In the case of a centric fission, in addition to this kind of rearrangement we should also consider (1) the occurrence of the euchromatinization of the interstitial, heterochromatic C band that accompanies the subtelocentric W of both the clades and (2) that females of Ócsa possess a complex sex chromosome system of type $Z_1Z_2W_1W_2$. However, neither centric fissions nor euchromatinization of heterochromatin events have so far been noticed among lacertids (Olmo *et al.*, 1993), while a $Z_1Z_2W_1W_2$ sex chromosome system would constitute a novelty not only among lacertids but in vertebrates. Lastly, in the case of introgression of *carniolica* maternal materials within a viviparous genome, this may only spring from hybridation between females of *carniolica* with viviparous males; in this case the resulting offspring should be compulsory oviparous, since cross breeding experiments between oviparous and viviparous strains of *L.vivipara* showed that the parental female dictate the reproductive mode of the offspring (Arrayago *et al.* 1996).

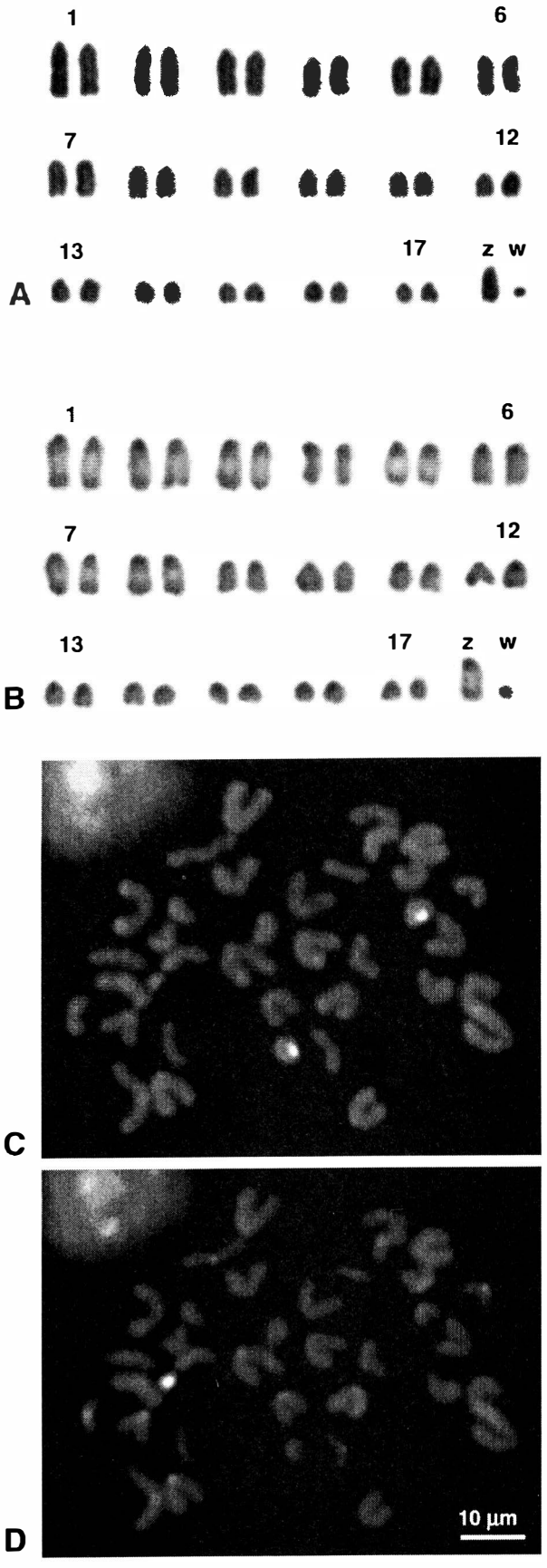

FIG. 2. Giemsa stained (A) and C-banded (B) karyotypes and a sequentially C-banding+ CMA_3 (C)+DAPI(D) stained metaphase plate of a female of *L. vivipara* from Ócsa.

In conclusion the adaptive value of viviparity (recently reviewed by Blackburn 2000) is further supported by our results, which point to an independent origin of viviparity in Ócsa and elsewhere in Europe.

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