

Intraspecific Phylogeography of *Lacerta vivipara* and the Evolution of Viviparity

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The lacertid lizard *Lacerta vivipara* is one of the few squamate species with two reproductive modes. We present the intraspecific phylogeny obtained from neighbor-joining and maximum-parsimony analyses of the mtDNA cytochrome *b* sequences for 15 individuals from Slovenian oviparous populations, 34 individuals from western oviparous populations of southern France and northern Spain, 92 specimens from European and Russian viviparous populations, and 3 specimens of the viviparous subspecies *L. v. pannonica*. The phylogeny indicates that the evolutionary transition from oviparity to viviparity probably occurred once in *L. vivipara*. The western oviparous group from Spain and southern France is phylogenetically most closely related to the viviparous clade. However, the biarmed W chromosome characterizing the western viviparous populations is an apomorphic character, whereas the uniarmed W chromosome, existing both in the western oviparous populations and in the geographically distant eastern viviparous populations, is a plesiomorphic character. This suggests an eastern origin of viviparity. Various estimates suggest that the oviparous and viviparous clades of *L. vivipara* split during the Pleistocene. Our results are discussed in the framework of general evolutionary models: the concept of an oviparity–viviparity continuum in squamates, the cold climate model of selection for viviparity in squamates, and the contraction–expansion of ranges in the Pleistocene resulting in allopatric differentiation. © 2001 Academic Press

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INTRODUCTION

The evolutionary shift from an oviparous to a viviparous reproductive mode, which is likely to have a pronounced effect on the reproductive success and the fitness of the organisms concerned, is a biological event of interest. Squamate reptiles are of particular interest for the study of this evolutionary shift as it has been shown that viviparity has evolved far more often in squamate reptiles (more than 100 times) than in all other lineages of vertebrates (34 times) (Blackburn, 1992, 1999).

The intriguing suggestion that the reverse transition from viviparity back to oviparity may also have occurred frequently in squamate reptiles has resulted in a vigorous debate. However, the reverse transition documented by Benabib *et al.* (1997) is likely to be erroneous, because these authors did not correctly assess the reproductive mode of some of the taxa they studied (Mendez-De La Cruz *et al.*, 1998) and the reverse transitions suggested by other studies (De Fraipont *et al.*, 1996, 1999) have also been criticized, in particular, because of uncertainties in the phylogenies they employed (Shine and Lee, 1999; Blackburn, 1999). Nevertheless, all these authors recognize that the reversion from viviparity cannot be ruled out on theoretical grounds. Therefore, further empirical research is needed. To avoid erroneous conclusions, empirical research aimed at polarizing the evolutionary transitions between reproductive modes should be interpreted in the context of the phylogenetic relationships of the taxa. Species with reproductive bimodality, that is the coexistence of oviparous and viviparous populations within a single species, represent ideal models for in-

vestigating this problem. Indeed, intraspecific phylogenies are less subject to reconstruction artifacts (e.g., long branch attraction; Sanderson *et al.*, 2000) than high level phylogenies. Furthermore, in high-level phylogenies, the problem of missing (extinct) intermediate clades can be misleading in polarizing evolutionary transitions. Reproductive bimodality has been documented for three species of squamates: the Australian scincid lizards, *Lerista bougainvilli* and *Saiphos equalis* (Fairbairn *et al.*, 1998; Qualls, 1996; Qualls *et al.*, 1995; Qualls and Shine, 1998; Smith and Shine, 1997), and the Eurasian lacertid lizard, *Lacerta vivipara*.

In *L. vivipara*, viviparous populations occur from central France and the British Isles up to Scandinavia and eastern Russia. Oviparous populations of *L. vivipara* were first identified in the extreme southwest of the range, from the Cantabrian mountains in Spain up to the Pyrenees and Aquitaine regions in southern France (Lantz, 1927; Braña and 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 nonfunctional oviparous structures); similarity in morphology, ecology, allozymes, and mtDNA; and successful experimental (laboratory) hybridizations all suggest that oviparous and viviparous populations of *L. vivipara* are very closely related and, hence, are likely to have diverged only recently (Arrayago *et al.*, 1996; Bea *et al.*, 1990; Guillaume *et al.*, 1997; Heulin, 1990; Heulin *et al.*, 1992, 1993, 1999). A biogeographic scenario, based on Pleistocene events, may account for this recent evolution, and for the geographic distribution of the two reproductive forms (Heulin *et al.*, 1993). During the Quaternary glaciations, the original populations (presumably still oviparous) may have retreated in two directions: southwest and southeast. Viviparity evolved and rapidly propagated among the southeastern populations, somewhere between the Balkan Peninsula and the south of Russia, whereas the southwestern populations remained oviparous in the Pyreneo-Iberian region. During interglacial periods (including the present postglacial period), the newly evolved viviparous populations would have progressively (re)colonized northeastern and northwestern countries, without any secondary contact with the residual oviparous populations isolated in the southwestern (Pyreneo-Iberian) refugium (Heulin *et al.*, 1993).

This scenario, suggesting that viviparity was rapidly selected during a period of cold climate conditions, is in accord with a widely accepted theory ("cold climate theory") for the selective pressures associated with the evolution of viviparity in squamates. Moreover, the time scale of this scenario is in accord with our allozymes and mtDNA analyses which both suggest that the differentiation of the two reproductive forms of *L.*

vivipara could have begun between 2 and 0.5 mybp, that is, during the Pleistocene (Guillaume *et al.*, 1997; Heulin *et al.*, 1999). Finally, some aspects of this scenario are also consistent with a karyological variation identified in *L. vivipara*: females from southwestern oviparous populations and from eastern viviparous populations both have an uniarmed (acrocentric-subtelocentric) W chromosome, whereas those from western viviparous populations possess a biarmed (metacentric) W chromosome (Odierna *et al.*, 1998; and Fig. 1). Assuming that oviparity corresponds to the ancestral (pleisomorphic) reproductive mode in squamates (see above and Discussion), then the presence of an "oviparous-like" (i.e., uniarmed) W chromosome in the eastern viviparous populations effectively supports the hypothesis that the center of origin of viviparity of *L. vivipara* should be eastern. However, until recently, no residual oviparous populations had been found in this geographic area. Therefore, the very recent discovery of oviparous populations of *L. vivipara* in Slovenia (Heulin *et al.*, 2000) reactivates the question. Could these Slovenian populations be considered as belonging to the oviparous lineage from which viviparity arose?

Molecular phylogeography (Avise *et al.*, 1987) is an appropriate tool to investigate the evolution of viviparity and the biogeographic history of *L. vivipara*. In a preliminary study, Heulin *et al.* (1999) investigated the relationships between some oviparous and viviparous populations using 16S rDNA mtDNA and showed the existence of a major phylogeographic break between the oviparous and viviparous populations of *L. vivipara*. However, the level of variation of the 16S rDNA sequences was too low to fully resolve relationships between the two forms and several key hypotheses may be critically tested by the use of a faster evolving gene (cytochrome *b*) and a much more comprehensive sample.

The sample in this study includes several important groups that were not previously taken into account: the eastern viviparous group (i.e., with uniarmed W chromosome), the oviparous group recently discovered in Slovenia (Heulin *et al.*, 2000), and the viviparous subspecies *L. v. pannonica* (Lac and Kluch, 1968) inhabiting some lowlands of central Europe (eastern Austria, northern Hungary, and southern Slovakia). We use these data to determine if the eastern viviparous group (with uniarmed W) and the western viviparous group (with biarmed W) are a single viviparous clade or if viviparity arose twice. We also establish the phylogenetic position of Slovenian populations and the subspecies *L. v. pannonica*.

MATERIALS AND METHODS

Tissue samples were obtained from specimens conserved in 95% ethanol. Two lacertid species, *Lacerta*

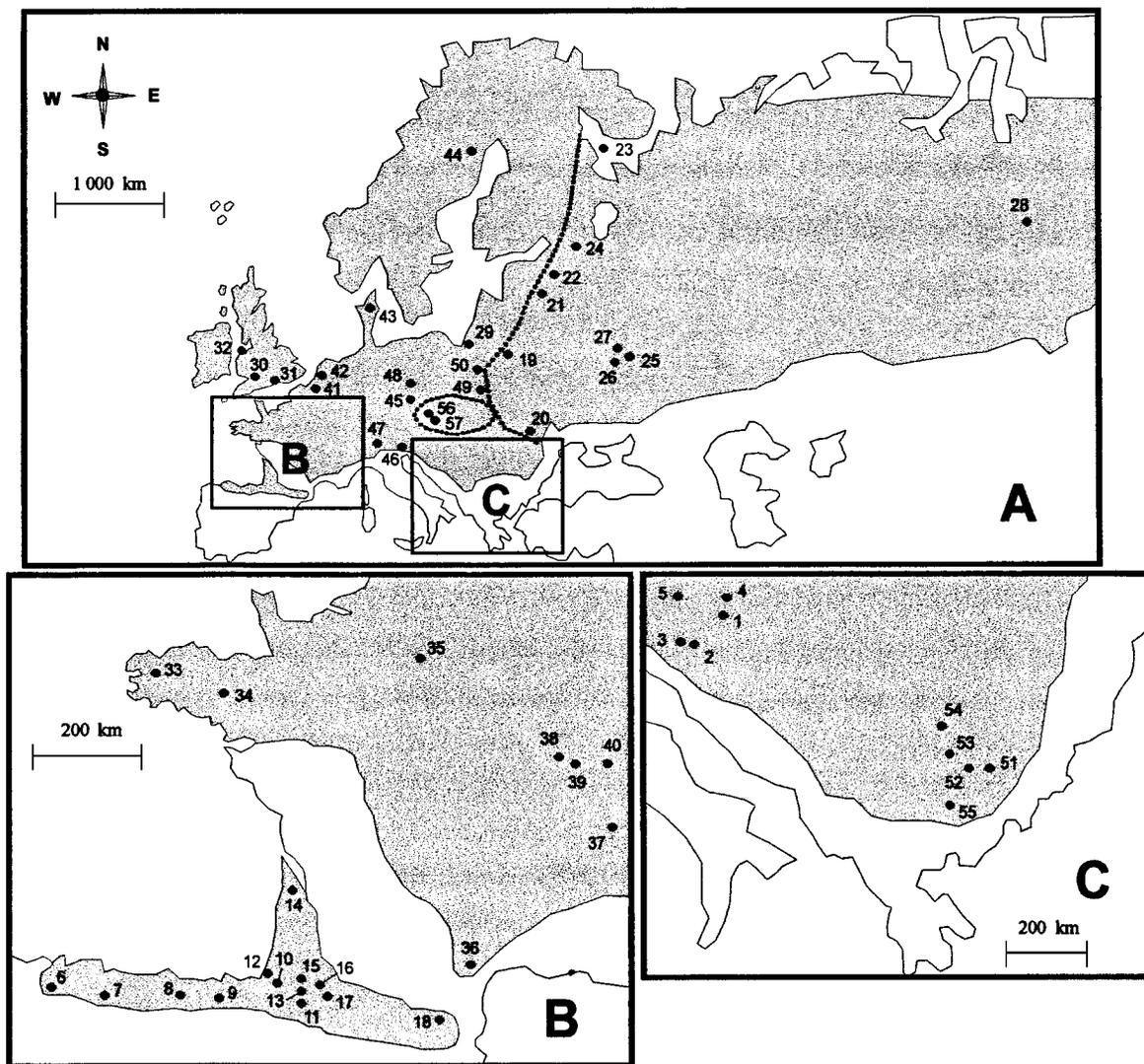


FIG. 1. Localities of the sampled populations. General map (A) and detailed map for southwestern Europe (B) and the Balkan peninsula (C). Population codes as in Table 1: 1 to 5 Slovenian oviparous group, 6 to 18 western oviparous group, 19 to 29 eastern viviparous populations, 30 to 55 western viviparous group, and 56 and 57 populations of the viviparous subspecies *L. v. pannonica*. The dashed lines are the geographic limits (after Kupriyanova, 1990; Odierna *et al.*, 1998) between the western viviparous group with a biarmed W chromosome, the eastern viviparous group with an unarmed W chromosome, and the subspecies *L. v. pannonica* which also possess a unarmed W.

bilineata (from Paimpont, France) and *Podarcis muralis* (from Souprosse, France), were used as outgroups in our analysis. Our ingroup data set (list of origin and localization in Table 1 and Fig. 1) consisted of 34 specimens from the western (French and Spanish) oviparous group, 15 specimens from the Slovenian oviparous group, 74 specimens from the western viviparous group (with biarmed W chromosome), 18 specimens from the eastern viviparous group (with unarmed W), and 3 specimens from the subspecies *L. v. pannonica* which is viviparous (personal observation) and also possesses an unarmed W chromosome (Kupriyanova and Böhme, 1997). All the sequences used in our study are deposited in the GenBank database under Accession No. AF247976 to AF248007.

Total DNA was chelex extracted according to Estoup *et al.* (1996) from small amounts of tail. A 429-bp segment of mtDNA (406 bp from cytochrome *b* and 23 bp from the adjacent Glu-tRNA genes; Fig. 2) was amplified using primers MVZ04 and MVZ05 (Smith and Patton, 1991). Sequencing of double-stranded DNA was performed in both directions using a Big Dye terminator cycle sequencing kit (Perkin-Elmer Biosystems) using the manufacturer's instructions and sequences were run on an ABI 310 genetic analyzer (Perkin-Elmer Biosystems). Sequence were aligned using Sequencher and phylogenetic analyses were performed with PAUP* version 4.0b4 (Swofford, 1999) using maximum-parsimony (MP, heuristic search and 100 random addition of sequences) and neighbor-joining (NJ

TABLE 1

Origin of Samples, Population Code, Sample Size, and Codes of the Haplotypes Identified

Country	Locality	Population code	Sample size	Haplotype	
Oviparous populations					
Slovenia	Medvece-Dravsko Pole	1	1	OS1	
	Cerknisko Jezero	2	3	OS1	
	Rakov Skocjan	3	3	OS1	
	Pohorje-Kot	4	4	OS1	
	Podkoren-Zelenci	5	4	OS1	
Spain	Puerto de Letariegos	6	1	OC4	
	Puerto de Tama	7	1	OC3	
	Alto de Tornos	8	2	OC2	
	Alto de Barazar	9	1	OC2	
France	Irati	10	2	OC1	
	Pourtalet	11	5	OC1	
	Moura de Montrol	12	2	OC5(1)/OF1(1)	
	Gabas	13	7	OC1(6)/OF1(1)	
	Saint Raphaël	14	2	OF1	
	Louvie	15	4	OF1(2)/OF2(2)	
	Plateau de Ger	16	1	OF3	
	Col des Palomnières	17	3	OF1	
Pinet-Bélesta	18	3	OF4		
Viviparous populations					
Bielorussia	Grodno	19	1	VU1	
Romania	Mures county	20	5	VU4	
Russia	Pskov	21	1	VU2	
	Loga	22	1	VU2	
	Srednii island	23	1	VU1	
	Krasnieti	24	1	VU1	
	Tchekchov	25	2	VU2	
	Borovsk	26	1	VU1	
	Volokolamsk	27	2	VU1	
	Turukchanskii Krai	28	3	VU1(2)/VU3(1)	
	Rybachii	29	2	VB11	
	England	Bristol	30	5	VB1
		Winchester	31	4	VB1(3)/VB2(1)
		Anglesey	32	3	VB1
	France	Saint Rivoal	33	2	VB1
		Paimpont	34	4	VB1(2)/VB3(2)
Rambouillet		35	1	VB4	
Mas de la Barque		36	3	VB1	
Chambery		37	2	VB1	
Bonnevaux		38	6	VB5(5)/VB6(1)	
Switzerland	Lac de Joux	39	1	VB1	
	Chatel Saint Denis	40	1	VB1	
Belgium	Kalmthout	41	4	VB1	
Netherlands	Overasseltse-Haterste	42	1	VB1	
Denmark	Jutland	43	1	VB1	
Sweden	Umea	44	1	VB1	
Czech Republic	Trebon	45	1	VB1	
Italy	Lago Casera	46	1	VB8	
	Chiareggio	47	1	VB7	
	Szklarska Poreba	48	5	VB1	
Poland	Ustrzyki Gorne	49	5	VB9	
	Krutyn	50	5	VB10	
	Rila-Beli Iskar	51	3	VB11	
Bulgaria	Rila-Govedarci	52	3	VB12	
	Vitocha	53	2	VB13	
	Balkan-Petrohan	54	4	VB11	
	Pirin	55	3	VB14(2)/VB15(1)	
Viviparous subspecies <i>L. v. pannonica</i>					
Austria	Moosbrunn	56	1	PA1	
Border Austria-Hungary	Fërto Lake	57	2	PA1	

Note. When different haplotypes are present in one population, the number of individuals carrying each haplotype is given between parentheses.

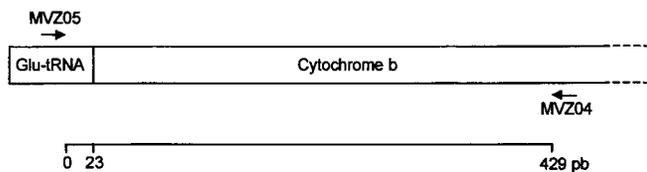


FIG. 2. Location of primers used and length of the DNA fragment analyzed.

with Juke and Cantor distances) methods. Statistical support of nodes was estimated by bootstrapping (1000 replicates). Once the monophyly of *L. vivipara* haplotypes had been verified, we used the most basal haplotype of this species to root the phylogenetic trees. This procedure which reduces homoplasy due to the use of distant outgroups may enhance the resolution of the phylogeny (Smith, 1994; Castelloe and Templeton, 1994).

Net nucleotide divergence (Da) between geographic groups was calculated from pairwise distances (Jukes and Cantor) between haplotypes, using DnaSP version 3.14 (Rozas and Rozas, 1999). This metric, which corresponds to the between-group variation corrected for within-group variation in haplotypes, can be used in calculating the splitting time of the groups (Nei, 1987). To estimate these splitting times from Da values, we used various published evolutionary rates (1 to 4.1% per million years) for different lizards species (Thorpe *et al.*, 1993, 1994; Guebitz *et al.*, 2000; Malhotra and Thorpe, 2000). The highest rate (4.1%) for cytochrome *b* divergence was calculated by us from the net nucleotide divergence of 0.33% between two insular populations of *Podarcis atrata* (populations CG and MA from Castilla *et al.*, 1998) whose separation time is 80,000 years (Hernandez-Pacheco and Asensio, 1966). This upper rate may be relatively unreliable as it is based on so few base-pair substitutions.

RESULTS

Cytochrome b Sequence Variation

The 429 bp were aligned including 132 variable positions; no indel was detected in the coding region (406 bp) where 116 substitutions occurred with 21 amino acid changes (outgroups included). The mean base composition was A, 0.29; C, 0.24; G, 0.12; and T, 0.35; and the transition/transversion ratio was 2.7 with the outgroups and 5.6 without the outgroups. Distances between haplotypes varied between 0.2 and 6.3% within the ingroup data set and up to 22.2% when the outgroups were considered. The sequence alignment and the pairwise distance matrix are available from the authors on request.

Phylogenetic Relationships

Using maximum-parsimony reconstruction with *P. muralis* and *L. bilineata* as outgroups, we obtained 15

equally parsimonious trees of 183 steps (consistency index CI = 0.8361, retention index RI = 0.8438, rescaled consistency index RC = 0.7054). The monophyly of *L. vivipara* is strongly supported (bootstrap value $P = 100$ both for MP and NJ analysis; see strict consensus tree and NJ tree in Figs. 3a and 3b). Using the most basal haplotype of *L. vivipara* (i.e., OS1 of the Slovenian oviparous group) as outgroup, we obtained 15 equally parsimonious trees of 65 steps (CI = 0.8615, RI = 0.9362, RC = 0.8065). This second procedure, which enhanced the resolution of the phylogeny (see further important nodes resolved in Fig. 3), did not modify the topologies of the strict consensus and of the NJ trees.

Five different clades can be distinguished. The first dichotomy is found between the haplotype OS1 (clade A, characterizing all the Slovenian oviparous populations) and a second clade composed of all other haplotypes. The monophyly of this second clade is not well supported by parsimony analysis, but is supported by NJ analysis ($P = 75$) and also by chromosomal data (see Discussion). In this second clade, four haplogroups can be distinguished: clade B, the western oviparous group (populations 6 to 18 from southwestern France and northwestern Spain, with haplotypes OC1 to OC5 and OF1 to OF4; Table 1 and Fig. 1); clade C, the haplotype (PA1) observed in the three specimens of *L. v. pannonica* (Austrian lowland population 56–57 in Table 1 and Fig. 1); clade D, the eastern viviparous group in which the W chromosome is uniarmed (populations 19 to 28 from Bielorrussia, Russia, and Romania, with haplotypes VU1 to VU4; Table 1 and Fig. 1); and clade E, the western viviparous group in which the W chromosome is biarmed (populations 29 to 55, with haplotypes VB1 to VB15; in Table 1 and Fig. 1).

The monophyletic nature of the viviparous group (clade C + D + E) is not supported by the MP consensus tree because of the unresolved position of *L. v. pannonica*. However, the NJ analysis suggests that this group could be monophyletic (bootstrap $P = 53$). The monophyly of the western + eastern viviparous group (clade D + E) is suggested both by parsimony and NJ analyses ($P = 65$ and $P = 64$, respectively).

Within clade B, which comprises all the haplotypes observed in the oviparous populations from southern France and northern Spain, two phylogeographic groups can be distinguished (Figs. 3a and 3b): a southwestern group (clade B1, $P = 62$ in MP, $P = 87$ in NJ) corresponding to the haplotypes (OC1 to OC5) observed in the oviparous populations extending from the Cantabrian mountains up to western Pyrenees (populations 6 to 13 in Table 1 and Fig. 1) and a northeastern group (clade B2, $P = 71$) corresponding to the haplotypes (OF1 to OF4) observed in the populations from the north of Aquitaine and from the central and eastern Pyrenees (populations 12 to 18). On the geographic boundary between these northeastern and southwest-

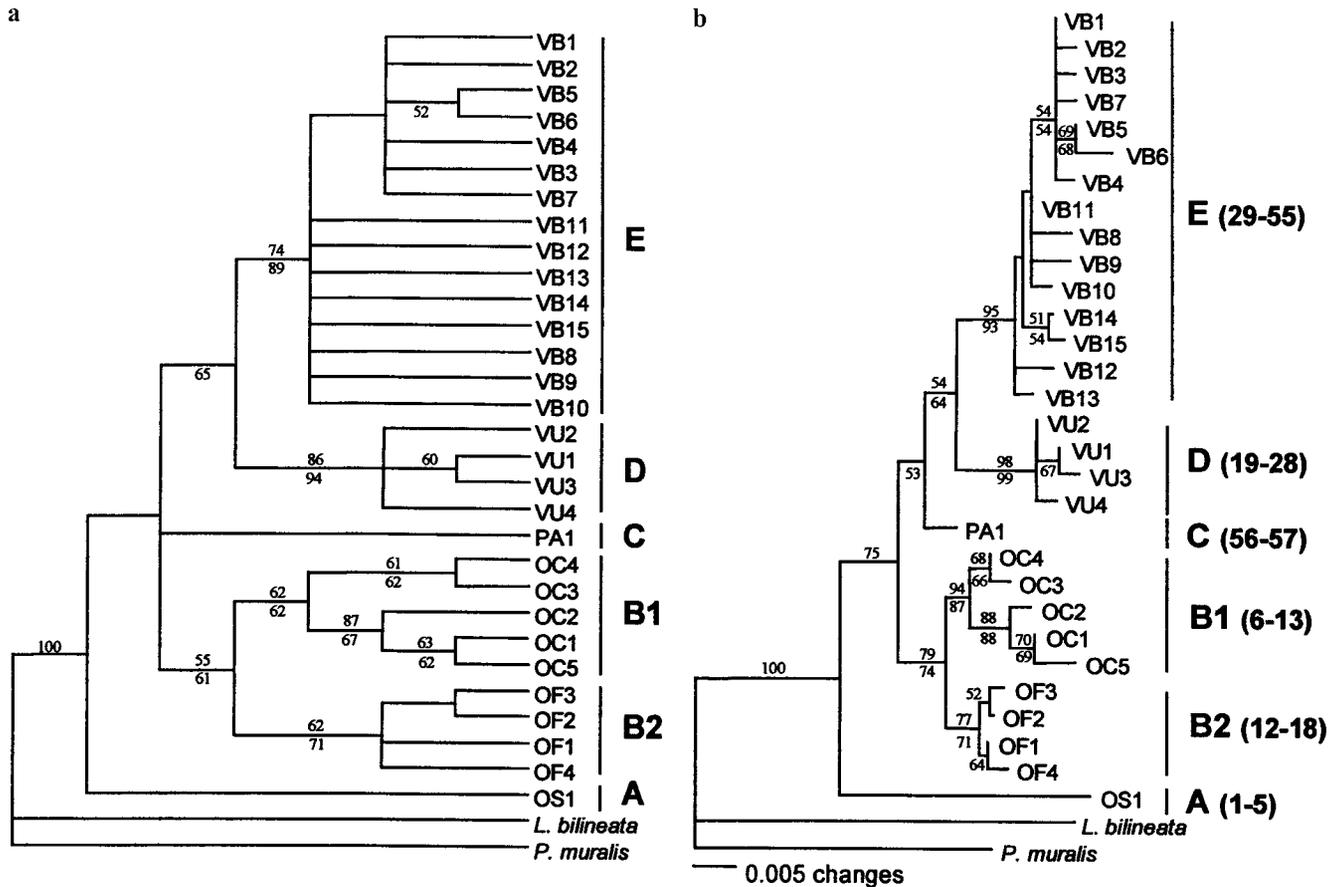


FIG. 3. Phylogenetic trees of *Lacerta vivipara*, obtained from parsimony (a) and neighbor-joining (b) analyses. Bootstrap values exceeding 50% are presented: values obtained with *Lacerta bilineata* and *Podarcis muralis* as outgroups (above branches) and values obtained with the OS1 haplotype as outgroup (below branches). The main groups identified are indicated on the right of the trees: the Slovenian oviparous (Clade A), the western oviparous (Clade B1 + B2), the viviparous subspecies *L. v. pannonica* (C), the eastern viviparous (clade D), and the western viviparous (clade E) groups. The numbers between parentheses represent population codes (as in Fig. 1).

ern haplogroups, we found two oviparous populations which possess haplotypes of the two clades (i.e., coexistence of haplotypes OF1 and OC5 in population 12 and of haplotypes OF1 and OC1 in population 13; Table 1 and Fig. 1).

Net Nucleotide Divergence and Divergence Times of Clades

The net nucleotide D_a , and the corresponding estimate of splitting time, of the major clades are presented in Table 2. The haplotype of the subspecies *L. v. pannonica* was not taken into account for calculation of divergence between western oviparous and viviparous groups, because of the uncertainty about its phylogenetic position.

DISCUSSION

The Phylogenetic Position of the Oviparous Populations from Slovenia

The biogeographic scenario presented in the Introduction hypothesizes that the Slovenian oviparous

populations belongs to—or is the closest extant relative of—the oviparous lineage from which viviparity arose. This hypothesis is rejected: our study estab-

TABLE 2

Net Nucleotide Divergence (D_a and 95% Confidence Intervals) and Estimated Splitting Times between Clades

Divergence between clades	D_a (95% CI)	Splitting time (mybp)	
		A	B
A vs (B + C + D + E)	4.2 (2.2–6.2)	1.02 (0.54–1.51)	4.2 (2.2–6.2)
B vs (D + E) ^a	1.8 (1.0–2.6)	0.44 (0.24–0.63)	1.8 (1.0–2.6)
D vs E	1.9 (1.1–2.7)	0.46 (0.27–0.66)	1.9 (1.1–2.7)
B1 vs B2	0.9 (0–1.9)	0.22 (0–0.46)	0.9 (0–1.9)

Note. Clades as in Fig. 3. The splitting times were estimated using a divergence rate of 4.1% (A) or of 1% (B) (see Materials and Methods).

^a Given its uncertain phylogenetic position, we did not take into account the subspecies *L. v. pannonica*, for calculating the D_a and the splitting time of the western oviparous vs viviparous clades.

lishes that the single haplotype (OS1) observed in all the oviparous populations of Slovenia clearly branches off at the base of the phylogenetic tree, before all other oviparous and viviparous lineages of *L. vivipara*.

This result, obtained from analysis of mtDNA sequences, is congruent with karyological data. It has long been stressed that a particular karyological event occurred in *L. vivipara*: an unequal number of chromosomes in males and females (respectively $2N = 36$ and 35 chromosomes), which has been documented for the viviparous populations and for the western oviparous populations of this species, has been interpreted as the result of a fusion of an ancestral W chromosome with an autosome, giving rise to a neo-W and to a particular female sex chromosome system called Z1Z2W (Chevalier *et al.*, 1979; Odierna *et al.*, 1993, 1998; Belcheva *et al.*, 1986; Kupriyanova *et al.*, 1995; Kupriyanova and Rudi, 1990; Kupriyanova 1986, 1990; Kupriyanova and Böhme, 1997). However, the evolutionary stage just prior to the W + autosome fusion, (i.e., females with $2N = 36$ chromosomes) had not been discovered until very recently. Our recent karyological investigations reveal that females from all the Slovenian oviparous populations exhibit a chromosomal formula ($2N = 36$) that corresponds to the ancestral evolutionary stage (Odierna *et al.*, 2001).

The fact that karyological and mtDNA analyses both indicate that the Slovenian oviparous group branches off before the western (French–Spanish) oviparous group and the fact that these two oviparous groups exhibit reproductive differences (Heulin *et al.*, 2000) provide a rare example of the oviparity–viviparity continuum (Shine, 1983; Qualls *et al.*, 1997). Presumably the evolutionary shift from oviparity to viviparity in squamates proceeds through a gradual increase in the length of time eggs are retained *in utero* prior to oviposition; that is through a gradual increase in the developmental stage reached by the embryos at oviposition, which results in a gradual shortening of the subsequent incubation period of the eggs.

We observed that the eggs laid by females of the Slovenian clade contain embryos significantly less developed at the time of oviposition and require significantly longer incubation period before hatching than the eggs laid by females of the western (French–Spanish) oviparous clade (Heulin *et al.*, 2000). In accord with the positions of these two clades in our phylogenetic tree, Slovenian and Spanish–French oviparous clades of *L. vivipara* might thus represent respectively a earlier and a later stage on an oviparity–viviparity continuum.

Phylogeography of the Western (French and Spanish) Oviparous Populations

Our study reveals that the western oviparous populations, from southwestern France and northern Spain, form a monophyletic clade separate from the

Slovenian oviparous clade. This western oviparous clade is nevertheless composed of two haplogroups which have distinct geographic distributions: a southwestern group (haplotypes OC1 to OC5 populations 6 to 11 in Table 1 and Fig. 1) and a northeastern group (haplotypes OF1 to OF4; populations 14 to 18 in Table 1 and Fig. 1).

The geographic variation of cytochrome *b* haplotypes among the western oviparous populations parallels those of the *MPI* alleles (mannose phosphate isomerase enzyme): the populations from Aquitaine (including population 14) and from the eastern, central, and northwestern parts of the Pyrenean range (including our populations 15 to 18) exhibit exclusively the fast-migrating alleles *MPI*^{110–120}, whereas the populations from the Cantabrian mountains and the southwestern Pyrenees (populations 6 to 11) exhibit exclusively the slow migrating allele *MPI*⁹⁰ (Guillaume *et al.*, 2000). Furthermore, the two populations in which fast migrating and slow migrating *MPI* alleles coexist (Guillaume *et al.*, 2000) correspond to those populations for which our mtDNA study reveals the coexistence of the two groups of haplotypes (haplotypes OF1 and OC5 in population 12; haplotypes OC1 and OF1 in population 13). Therefore, both allozyme and mtDNA data suggest that the western oviparous clade is composed of two distinct phylogeographic groups that have come into contact in at least two populations. This phylogeographic pattern, corresponding to the category II of Avise *et al.* (1987), is characteristic of recent, secondary, admixture zones between populations that previously evolved in allopatry. Based on *MPI* alleles, Guillaume *et al.* (2000) previously hypothesized that two groups of oviparous populations could have retreated to different refugia (one in southern France and the other in northwestern Spain) during the Pleistocene glaciations and subsequently come into secondary contact in the vicinity of the western part of the Pyrenean range (the mountain separating France from Spain) during warmer post glacial periods. Suture zones (secondary contacts) between races, subspecies or sister species, have also been documented for a wide variety of animal and plant taxa in this biogeographic area (Hewitt, 1988; Salomon and Hemin, 1982; Mossakowsky *et al.*, 1990; Lazare, 1992; A1cobendas *et al.*, 1996). The existence of such suture zones may be related to the pattern of contractions/expansions of ranges during the Pleistocene glaciations, to refugia occupied during warming periods (Pyrenean and Cantabrian mountains) as well as during cold periods (Iberian and French Lowlands), and to the existence of geographic barriers (mountain glaciers oriented east–west on the Pyrenean and Cantabrian mountains) favoring allopatric differentiation during cold periods, but becoming more permeable and allowing secondary contacts during warming periods (for general discus-

sion on these subjects see Hewitt, 1996; Taberlet *et al.*, 1998; Thorpe, 1984).

Phylogeography and the Evolution of Viviparity of Lacerta vivipara

Viviparity certainly evolved from oviparity in many occasions in squamates, although the possibility that the reverse transition (from viviparity back to oviparity) may also have occurred is still in dispute (see Introduction and the recent controversy in De Fraipont *et al.*, 1996, 1999; Shine and Lee, 1999; Blackburn, 1999). The topology of the phylogenetic trees of *L. vivipara* obtained in this study indicates that viviparity probably evolved only once in this species and that there is no evidence of reversal from viviparity. Some questions still remain, for example: where, and from which oviparous lineage, did viviparity arise? The existence of a plesiomorphic (uniarmed) W chromosome in both the western oviparous group and the viviparous populations from the central (subspecies *L. v. panonica*) and eastern (clade D) parts of the species' range strongly supports the hypothesis that viviparity did not arise from the western (French and Spanish) oviparous lineage, but rather from a more eastern oviparous lineage. Given that the Slovenian oviparous lineage cannot have played this role (see above), we must therefore posit that an eastern oviparous lineage with uniarmed W actually exists, but has not yet been discovered, or that this oviparous group became extinct.

Our phylogenetic trees confirm that the uniarmed form of the W chromosome is a plesiomorphic character and, therefore, that the biarmed form of the W chromosome corresponds to an apomorphic character which appeared after the evolution of viviparity. Kupriyanova and Rudi (1990) and Odierna *et al.* (1998) hypothesized that the karyological processes involved in this transition involved a pericentric inversion that gave rise to a W chromosome with two long arms. The biogeographic scenario of Heulin *et al.* (1993) also suggests that the western part of Europe may have been secondarily colonized by the viviparous populations that initially evolved further east. In addition to the chromosomal data, our phylogenetic analysis provides other arguments supporting the hypothesis of a recent colonization of the westernmost part of Europe by viviparous populations with biarmed W chromosome. Two groups can be distinguished within the viviparous populations with biarmed W (clade E in Fig. 3): a paraphyletic group corresponding to the easternmost populations of the clade E (haplotypes VB8 to VB15, populations 29, 46, and 49 to 55) and a monophyletic group corresponding to the western populations of this clade E (haplotypes VB1 to VB7, populations 30 to 45, 47, and 48). The basal position of the former group with respect to the second group suggests a progressive differentiation of the haplotypes from east to west within clade E.

Chronological and Adaptive Aspects

Our data suggest that four evolutionary shifts occurred successively during the radiation of *L. vivipara*: (1) a fusion of the W chromosome with an autosome which resulted in a shift from females with 36 chromosomes (as observed in the extant Slovenian lineage) to females with 35 chromosomes (as observed in all other lineages); (2) the evolution of viviparity; (3) a pericentric inversion of the W chromosome, which resulted in the shift from an uniarmed W to a biarmed W within the viviparous clade; and (4) the differentiation of two subgroups within the western (French and Spanish) oviparous clade (Fig. 4).

According to our estimates of splitting times (see Table 2), all these events (except perhaps the divergence of the Slovenian lineage) could have occurred during the Pleistocene. Such estimates of divergence time should of course be considered with caution. In fact, ideally, they should have been calculated by using a divergence rate of the cytochrome *b* gene of the species studied. We actually attempted to calculate such a divergence rate by comparing the haplotypes observed in England populations and in the closest populations living on the continent, assuming that the rising sea level separated these two groups about 8000 years ago. Unfortunately, because of a low level of divergence ($D_a \leq 0$) between the two groups, we were unable to calculate a realistic rate of cytochrome *b* divergence. From our allozyme data and by using a wide range of a calibration rates, we previously estimated the divergence time of the western oviparous and western viviparous groups of *L. vivipara* at between 0.5 and 2 mybp (Guillaume *et al.*, 1997; Heulin *et al.*, 1999). The congruence of the estimates of divergence time obtained from data concerning two distinct genetic system (i.e., allozymes/mtDNA), which both suggest a radiation during the Pleistocene, makes the time scale proposed plausible.

The possibility that the radiation of *L. vivipara* and, especially the evolution of viviparity in this species, occurred during the glacial phases of Pleistocene is interesting.

Its verification would provide an empirical support of the "cold climate model" positing that cold climatic conditions are one of the most important selective forces acting in favor of the evolution of viviparity in squamates (for review see Shine, 1985; Heulin *et al.*, 1991, 1997).

With regard to this, it can also be noted that the cold climate model could also account for the fact that the extant oviparous populations of *L. vivipara* are restricted to regions (NW Spain, SW France, Slovenia) of relatively low latitude compared to those occupied by most of the viviparous populations of this species. However, some viviparous populations, such as those of Bulgaria, exist at latitudes and altitudes comparable to those of the Pyre-

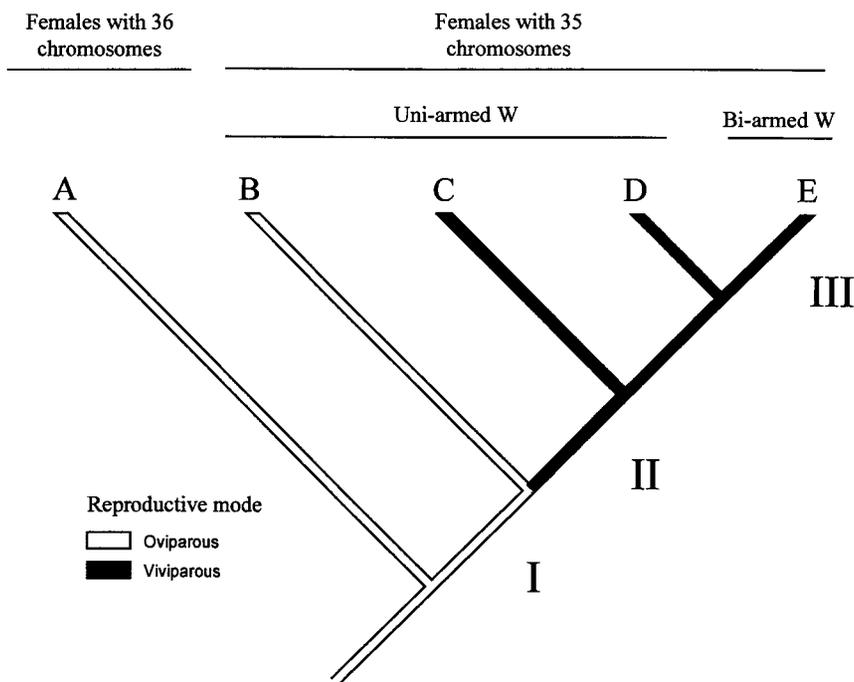


FIG. 4. Diagrammatic representation of the main evolutionary shifts in *L. vivipara*. Clades as in Fig. 3. The biological shifts are indicated on the right of the tree: I, translocation of W chromosome on an autosome (shift from females with $2N = 36$ to females with $2N = 35$ chromosomes); II, evolution of viviparity; III, pericentric inversion on the W chromosome (shift from a uniarmed to a biarmed W).

near oviparous populations (Guillaume *et al.*, 1997). Therefore, superior adaptation to the climatic conditions at lower latitude might not be the only factor explaining the persistence of oviparous populations in some southern regions. Fortuitous historical events, such as the emergence of local isolation barriers, could also have played a role in preventing contacts between the two reproductive forms and, hence, in favoring the local persistence of isolated oviparous populations in some southern regions. For example, the emergence, during the warmer postglacial period of the late Pleistocene, of ecological conditions (extension of dry Mediterranean biota in lowlands) that were unfavorable for *L. vivipara*, might have prevented a contact between the viviparous populations of the Massif Central and the isolated oviparous populations of the Pyrenees in southern France (Heulin *et al.*, 1993, 1997; Guillaume *et al.*, 2000). At the moment, our knowledge of the geographic extension of the Slovenian oviparous lineage is insufficient to assess whether similar events could have also led to geographic isolation of this group throughout the Pleistocene. However, it is worth noting that the Slovenian lineage is only represented by a single haplotype, which could be the result of a strong bottleneck in a small allopatric refuge population.

CONCLUSIONS AND PROSPECTS

The results of this study clearly underline the considerable interest in the use of *L. vivipara* as a model for

investigating numerous general evolutionary and biogeographic questions. With our current state of knowledge it is possible to propose a coherent phylogeographic and adaptive scenario integrating the concept of the oviparity–viviparity continuum, the model of allopatric differentiation associated with the contraction and expansion of ranges during the Pleistocene, and the cold climate model for the evolution of viviparity. Further research should attempt to improve and to test more thoroughly several aspects of this global scenario. In particular, we should improve our sampling in some regions (e.g., southeastern populations, Slovenian lineage, subspecies *L. v. pannonica*), and we should also attempt to investigate a more variable gene that would enable us to calculate divergence rate, from populations whose separation time is known.

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