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Variation in Eggshell Characteristics and in Intrauterine Egg Retention Between Two Oviparous Clades of the Lizard *Lacerta vivipara*: Insight Into the Oviparity–Viviparity Continuum in Squamates

Benoit Heulin,[1] Samuele Ghielmi,[2] Nusa Vogrin,[3] Yann Surget-Groba,[4] and Claude Pierre Guillaume[5]

[1]*UMR CNRS 6553, Station Biologique de Paimpont, 35380 Paimpont, France*
[2]*Civico Museo Insubrico di Storia Naturale, plazza Giovanni XXIII-21056 Induno Olona (Va), Italy*
[3]*Vransko 121, SI-3305 Vransko, Slovenia*
[4]*UMR CNRS 6553, Laboratoire de Parasitologie Pharmaceutique, Avenue du Professeur Léon Bernard, 35043 Rennes, France*
[5]*Laboratoire Biogéographie et Ecologie des Vertébrés, EPHE, 34095 Montpellier, France*

ABSTRACT The concept of the oviparity–viviparity continuum refers to the wide range in the length of intrauterine egg retention and, hence, in the stage of embryonic development at oviposition existing in squamates. The evolutionary process underlying this continuum may involve not only a lengthening of egg retention in utero, but also a marked reduction in the thickness of the eggshell. The idea that there may exist a negative correlation between the developmental stage reached by the embryo at oviposition and the eggshell thickness within squamates, although supported by the comparison of oviparous vs. viviparous species, has seldom been evaluated by comparing eggshell thickness of oviparous forms with different lengths of intrauterine egg retention. Eggs of two distinct oviparous clades of the lizard *Lacerta vivipara* were compared. The eggs laid by females from Slovenian and Italian populations have thicker eggshells, contain embryos on average less developed at the time of oviposition, and require a longer incubation period before hatching than the eggs laid by females from French oviparous populations. Our data and several other examples available from the literature support the idea that the lengthening of intrauterine retention of eggs and the shortening of the subsequent external incubation of eggs are associated with reduction in the thickness of the eggshell, at least in some lineages of oviparous squamates. The current hypotheses that may account for this correlation are presented and a few restrictions and refinements to those hypotheses are discussed. In particular, other changes, such as increased vascularization of the oviduct and of the extraembryonic membranes, may play the same role as the decrease of eggshell thickness in facilitating prolonged intrauterine egg retention in squamates. Future studies should also consider the hypothesis that the length of intrauterine retention might directly depend on the extent of maternal–fetal chemical communication through the eggshell barrier. J. Morphol. 252:255–262, 2002.

KEY WORDS: eggshell; oviparity; viviparity; squamates; *Lacerta vivipara*

The evolutionary shift from an oviparous (egg-laying) to a viviparous (live-bearing) reproductive mode is a biological event of importance because it likely altered the reproductive success of the organisms concerned. Squamate reptiles (i.e., lizards, snakes, and amphisbaenians) are of particular interest for the study of this evolutionary transition because viviparity has evolved far more often in squamates (100 times) than in all other lineages of vertebrates (34 times) (Blackburn, 1992, 1999). Moreover, several ideal models for comparative studies, that is, very closely related taxa (and even conspecific populations) that exhibit different reproductive modes, exist in squamate reptiles (Guillette, 1982; Heulin et al., 1991, 1993, 1997; Qualls et al., 1995; Smith and Shine, 1997; Lobo and Espinoza, 1999). Such models necessarily improve understanding of the oviparous/viviparous transition because they minimize the confounding effect of phylogenetic differences.

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*Correspondence to: B. Heulin, Station Biologique de Paimpont, 35380 Paimpont, France. E-mail: benoit.heulin@univ-rennes1.fr

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Squamates exhibit a wide reproductive diversity, well beyond a simple oviparity/viviparity dichotomy. In particular, the degree of embryonic development at oviposition varies considerably among oviparous squamates and ranges from species that lay eggs with a small embryonic disk (gastrula stage) to species that lay eggs containing almost fully developed embryos. This observation gave rise to the concept of the 'oviparity–viviparity continuum' (Packard et al., 1977; Shine and Bull, 1979; Shine, 1983; Blackburn, 1982; Xavier and Gavaud, 1986; Andrews and Mathies, 2000; also, see the debate between Blackburn, 1995, and Qualls et al., 1997). This concept posits that the evolutionary shift from oviparity to viviparity in squamates proceeds through a gradual increase in the time eggs are retained in utero prior to oviposition; that is, through a gradual increase in the developmental stage reached by the embryo at oviposition, which results in a gradual shortening of the subsequent incubation period of the oviposited eggs. In addition, the evolutionary process leading to the emergence of viviparity in Squamata consists not only in a lengthening of the egg retention in utero but also marked reduction in the thickness of the eggshell. Although numerous viviparous species of squamates still have an eggshell membrane enveloping the embryo during development, this structure never has a calcified layer and is always thinner (less than 10 μm) than the eggshell of oviparous species (Hoffman, 1970; Guillette and Jones, 1985; Stewart, 1985, 1990; Heulin, 1990; Guillette, 1993; Blackburn, 1993; Qualls, 1996). The most frequently proposed interpretation is that a thick eggshell slows fetal–maternal gas exchange and that this is incompatible with normal embryonic development in viviparous species, especially during the final growth phase of the embryo, when its oxygen requirement increases dramatically (Packard et al., 1977; Shine and Bull, 1979; Xavier and Gavaud, 1986; Qualls, 1996; Andrews and Mathies, 2000). An extension of this hypothesis has been proposed by Guillette (1991, 1993), who further hypothesized that a reduction in eggshell thickness may not only serve to augment gas exchanges, but may also facilitate the exchange of recognition factors between mother and embryo. According to Guillette (1991, 1993), a decrease in eggshell thickness could accelerate the diffusion of chemical signals from the embryo toward the mother and the subsequent interaction between those embryonic factors and the mother's tissue (uterus, corpora lutea) would then lead to an increase in the period of intrauterine egg retention.

The idea that the length of intrauterine retention of eggs (or the stage of embryonic development at oviposition) is negatively correlated with eggshell thickness within squamates, although supported by the comparison of oviparous vs. viviparous species (see above), has seldom been evaluated for intraspecific comparisons of oviparous forms with different lengths of intrauterine egg retention. To date, such intraspecific comparisons have been made only for the lizards *Lerista bougainvilli* (Qualls, 1996), *Sceloporus scalaris* (Mathies and Andrews, 1995), and *Lacerta vivipara* (Heulin et al., 1992; Arrayago et al., 1996; this study).

Lacerta vivipara is a lacertid lizard that has both oviparous and viviparous populations. Viviparous populations are widely distributed from the British Isles and central France into Scandinavia and eastern Russia. Geographically isolated oviparous populations of this species were first identified in the extreme southwest of the species' range, from the Cantabrian and Pyrenean mountains into the Aquitaine region in southern France. We successfully crossbred southwestern oviparous lizards with viviparous lizards from northwestern France and obtained F1 hybrids, which themselves bred and laid eggs in our laboratory (Arrayago et al., 1996). We showed that the eggs of the southwestern oviparous strain have an eggshell thicker (averaging 40 μm) than those of the eggs of F1 hybrids (about 25 μm) and that the viviparous embryo remains encased in very thin (less than 10μm) eggshell membrane up to parturition (Heulin, 1990; Heulin et al., 1992). We also showed that eggs of F1 hybrid females contained more developed embryos at oviposition and hatch more rapidly than the eggs laid by the southwestern oviparous females (Arrayago et al., 1996). Although this model is somewhat artificial (F1 hybrids obtained and bred in laboratory), it supports the idea that the lengthening of intrauterine retention of egg and the shortening of the subsequent external incubation of eggs is associated with reduction in the thickness of the eggshell in *L. vivipara*.

More recently, we identified another group of oviparous populations living in Slovenia and in northern Italy. This Sloveno-Italian oviparous group is geographically and phylogenetically distinct from the southwestern oviparous group previously identified. Analyses of the mtDNA cytochrome b gene sequences revealed that the Slovenian-Italian oviparous populations form a clade branching off at the base of the phylogenetic tree, whereas the southwestern oviparous clade and the viviparous clade occupy upper branches of this phylogenetic tree (Surget-Groba et al., 2001; unpublished data). In addition, the eggs laid by females from Slovenian and Italian populations contain embryos significantly less developed than those of the eggs laid by females from southwestern France (Heulin et al., 2000). Hence, it seems worth testing whether this difference in embryo stage at egg-laying is associated with a difference in eggshell thickness between the two groups.

Here we compare characteristics of the eggs (egg weight and embryo stage at egg laying, eggshell structure, and thickness) of a sample of oviparous Italian and Slovenian females to those of a sample of French oviparous females. We also present some

comparative data on incubation duration for subsamples of eggs of both groups that were incubated at the same temperature in our laboratory.

MATERIALS AND METHODS

Samples Studied and Laboratory Conditions

Lacerta vivipara is a small-sized (adults of 45–75 mm in snout-vent length) ground-dwelling lacertid, generally living in moist habitats. Detailed information on the reproductive cycle, life-history, and geographic distribution of its oviparous and viviparous forms have been published elsewhere (Heulin et al., 1991, 1997, 2000).

All the data presented here were obtained from oviparous females caught in natural populations after the mating period in late spring – early summer, 2000, and were kept for a short time (less than 1 month) in the laboratory, until they laid their first clutch. Data concerning the second clutches laid by some females were not considered in the present study because they were not sufficiently numerous to allow statistical comparisons.

The females were reared separately in plastic terraria. Each terrarium (30 × 20 × 20 cm) was equipped with a shelter, dishes of food and water, and a 40W bulb that provided heat for 6 h/day. The floor under the shelter was lined with damp sponge, to prevent clutches from drying. We checked terraria for clutches four times a day.

Clutches were obtained from 15 females from the Slovenian and Italian populations and from 26 females of the oviparous populations of southwestern France. These clutches were used to compare the stage of embryonic development at oviposition and the eggshell characteristics of the two groups.

Clutches from Slovenian populations were obtained from three females from Kot in the Pohorje Mountains (Lat. 46°26′ N, Long. 15°26′ E, Alt. 1,040 m), two females from Zelenci (46°30′ N, 13°44′ E, Alt. 840 m), two females from Cerknisko (45°46′ N, 14°22′ E, Alt. 480 m), one female from Ig (45°58′ N, 14°32′ E, Alt. 1,000 m).

The Italian sample was composed of one female from Tarvisio (46°30′ N, 13°36′ E, Alt. 800 m) and two females from Fusine (46°29′ N, 13°40′ E, Alt. 875 m) for the Frioule region in NE Italy, and one female from Oropa (45°37′ N, 7°58′ E, Alt. 1,180 m), one female from Lac Majeur (45°52′ N, 8°29′ E, Alt. 850 m) and two females from Varese (45°47′ N, 8°42′ E, Alt. 240 m) for the Piémont-Lombardian region in NW Italy.

Clutches from the French populations were obtained from one female from Col de Port (42°53′ N, 1°27′ E, Alt. 1,200 m), one female from Palomières (43°03′ N, 0°10′ E, Alt. 800 m), 13 females from Louvie (43°06′ N, 0°23′ W, Alt. 370 m), two females from Bénou (43°03′ N, 0°28′ W, Alt. 800 m), five females from Gabas (42°53′ N, 0°26′ W, Alt. 1,100 m), and four females from Pourtalet (42°47′ N, 0°24′ W, Alt. 1,800 m).

One egg per clutch was weighed and dissected immediately after being laid to determine the stage of embryonic development at oviposition following the staging tables (stages 1–40) of Dufaure and Hubert (1961). The egg contents (embryo and yolk) were removed and the eggshell was kept in 75° ethanol until it was processed for scanning electronic microscopy (SEM). The determination of the embryo stage at egg-laying and the SEM study of the eggshell thickness were performed for a single egg per clutch to ensure independence of the data. Indeed, previous observations revealed no difference in eggshell thickness or in the embryo stage at egg-laying among eggs of the same clutch (Heulin, unpublished data). The remaining eggs were either used for the incubation experiment (see below) or fixed in ethanol for other purposes. We did not attempt to incubate eggs from all the clutches obtained in our laboratory.

Data on incubation duration under standardized conditions are only available for a subset of 11 clutches of the Sloveno-Italian sample (all clutches from Slovenian populations, the two clutches from Fusine, and the one from Tarvisio) and of 11 clutches of the French sample (nine from Louvie, one from Gabas, one from Palomières). For these subsamples, 1–3 eggs from each clutch were incubated individually in small plastic containers. Each egg was placed on a filter paper overlaying damp (saturated) sand. We daily rehumidified the sand and the filter paper remained wet for the incubation period. The presence of condensation droplets inside the containers indicated that the relative humidity remained close to 100%. Incubation containers were kept in a temperature chamber providing a constant temperature of 22.5°C. This incubation temperature is within the daily range of temperature (12–28°C) and only slightly above the mean temperature (17–21°C) that we recorded in natural nests (Heulin et al., 1994). The incubation containers were checked daily for hatchling. We estimated the mean incubation duration for a given clutch by averaging the values of incubation duration observed for each individual egg of this clutch. Indeed, eggs from the same clutch hatched within 48 h in most (90%) cases.

Scanning Microscopy, EDS Analysis

Each eggshell was removed from 75° ethanol and cut longitudinally into several pieces using PASCHEFF-WOLFF's microsurgery scissors. These pieces of the eggshell were air-dried at room temperature and mounted with double-face Scotch tape on two brass stubs. The pieces stuck on one stub showed the outer surface of the eggshell (i.e., outer surface uppermost), whereas the pieces stuck on the

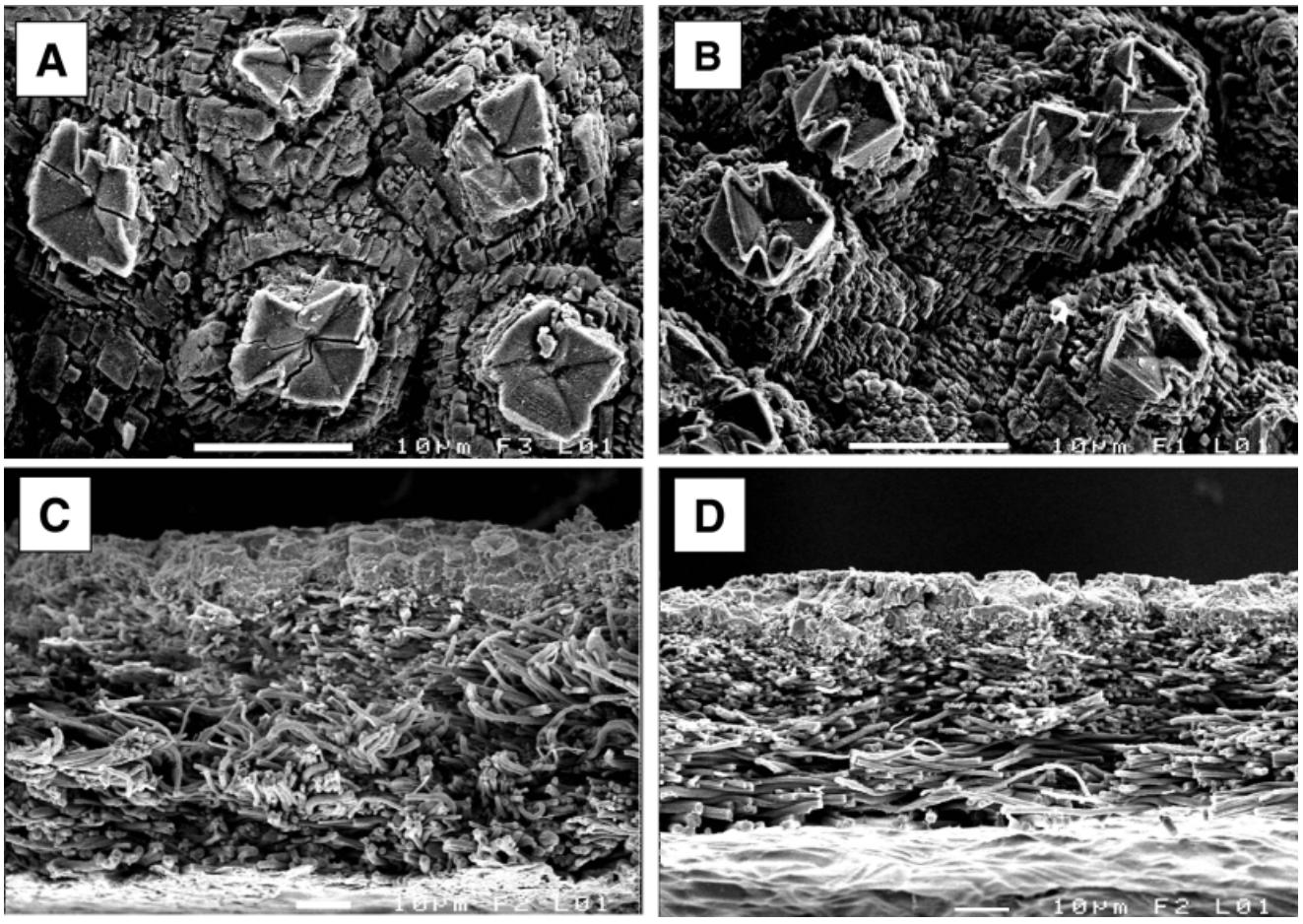

Fig. 1. Scanning electron photomicrographs of eggshells of oviparous *Lacerta vivipara*. Outer surfaces of a Slovenian egg (**A**) and of a French egg (**B**), showing the crystalline crust with both large and smaller crystals. Cross sections (outer edge uppermost) of a Slovenian eggshell (**C**) and of French eggshell (**D**), showing the fibrous layer overlain by the crystalline crust. Horizontal scale bars = 10 μm.

other stub showed cross sections of the eggshell (i.e., edges uppermost). All eggshell pieces were then coated with gold with a sputtering JEOL JFC 1000 and examined with a JEOL JSM 6301F SEM. Photos of the outer surface (×3,000) and of different cross sections (×1,000) were taken for each eggshell. The thickness of each eggshell was estimated by averaging 10 different measurements performed in different sites spaced along the length of 2–3 cross sections. We estimated the thickness of the fibrous layer, the thickness of the crystalline crust, and the total thickness (fibrous layer + crust) of each eggshell.

We determined the atomic composition of the crystalline crust of the eggshell for one egg from Slovenia and for one egg from France by means of an energy dispersive spectrometry (EDS) analysis. This analysis was performed on an OXFORD LINK ISIS spectrometer connected to a JEOL JSM 6400 SEM. The surface of the crust analyzed by EDS was 800 μm^2.

The possible presence of calcium carbonate was also tested by treating some shell pieces with dilute hydrochloric acid (effervescence test).

Statistical Analysis

All the mean values are presented ± 1 SD (standard deviation). Statistical tests were performed using MINITAB software. Mean values were compared using Student's *t*-test, with correction for unequal variances when necessary. We tested the possibility of correlation between some variables using Spearman's coefficient (rs) of correlation.

RESULTS

SEM observations did not reveal differences in eggshell structure between the two oviparous groups. In both cases the eggshell had a well-developed fibrous layer overlain by a relatively thin crystalline crust. The crystalline crust had large and

TABLE 1. Comparison of the egg characteristics, embryo stage of development at oviposition, and incubation duration between the French and the Sloveno-Italian oviparous groups of Lacerta vivipara

		French sample			Sloveno-Italian sample	
		N	Mean ± SD (range)		N	Mean ± SD (range)
Egg weight (mg) at oviposition	A	26	243.6 ± 31.2 (197–292)	ns	15	230.7 ± 31.9 (175–282)
	B	11	228.3 ± 31.1 (197–283)	ns	11	234.2 ± 24.5 (189–267)
Thickness of the fibrous layer of the eggshell (μm)	A	26	40.0 ± 8.0 (22–56)	***	15	64.4 ± 11.0 (52–87)
	B	11	41.4 ± 9.0 (22–56)	***	11	64.8 ± 12.4 (52–87)
Thickness of the crystalline crust of the eggshell (μm)	A	26	6.4 ± 1.2 (4–9)	*	15	7.5 ± 1.5 (5–11)
	B	11	6.7 ± 1.1 (5–9)	ns	11	7.7 ± 1.4 (6–11)
Total thickness of the eggshell (μm)	A	26	46.3 ± 8.2 (28–62)	***	15	72.0 ± 11.0 (62–94)
	B	11	48.2 ± 9.1 (28–62)	***	11	72.5 ± 12.2 (62–94)
Embryo stage at oviposition	A	26	33.1 ± 1.1 (30–35)	***	15	31.0 ± 0.8 (30–32)
	B	11	33.3 ± 1.3 (31–35)	***	11	31.2 ± 0.8 (30–32)
Incubation duration at 22.5°C (days)	B	11	28.6 ± 2.2 (25–33)	***	11	34.7 ± 1.8 (33–39)

A: clutches in which one egg was weighed and dissected just after oviposition to determine the embryo stage of development (according to Dufaure and Hubert, 1961) and to measure the eggshell thickness.
B: Subsample of clutches (11 from southwestern France, 11 from Slovenia and Italy) whose incubation duration at 22°5 was studied.
Comparisons of the two samples by Student's *t*-tests: difference nonsignificant (ns), significant at $P < 0.05$ (*) or at $P < 0.001$ (***).

angular crystals separated by a dense arrangement of smaller—often rhombohedral—crystals (Fig. 1).

EDS analyses were performed on surfaces of 800 μm^2 including both types of crystals. These analyses revealed an atomic composition of 21.7% Ca + 18.5% C + 59.4% O + 0.4% Mg for the crystalline crust of a Slovenian egg, and 20.2% Ca + 17.8% C + 61.7% O, + 0.3% Mg for the crystalline crust of a French egg. This indicates that the crystalline crust is an almost pure $CaCO_3$ with only minor traces of Mg. The presence of calcium carbonate was also confirmed by the fact that the crystalline crust readily effervesced when treated with hydrochloric acid.

Data on the egg characteristics at oviposition are summarized in Table 1. This table also presents data separately concerning the subsamples (11 clutches for each group) for which some eggs were incubated at 22.5°C. Egg weight at oviposition did not differ between the Sloveno-Italian populations and the French populations. The eggshell (fibrils + crust) was much thicker in the Sloveno-Italian sample than in the French sample. This difference in eggshell thickness between the two groups was, however, much more pronounced for the fibrous layer (difference of about 24 μm; significant both for the whole sample and for the subsample of incubated clutches) than it is for the crystalline crust (difference of about 1 μm; significant only for the whole sample). The range of embryonic development stages observed at oviposition in the French sample (stages 30–35) was greater than those of the Sloveno-Italian sample (stages 30–32). The embryos of the French sample are on average significantly more developed at oviposition than the Sloveno-Italian embryos (Table 1, Fig. 2). We found a significant negative correlation between eggshell thickness and the embryo stage of development at oviposition for the whole dataset (n = 41, rs = -0.58 significant at $P = 0.01$), but this correlation was not significant for the Sloveno-Italian sample (n = 15, rs = -0.27) and for the French sample (n = 26, rs = 0.07), considered separately. We did not find significant correlations between eggshell thickness and egg weight (n = 41, rs = -0.11), nor between egg weight and the embryo stage at egg-laying (n = 41, rs = 0.22).

The incubation duration of the French subsample of eggs kept at 22°5C was significantly shorter than those of the Sloveno-Italian eggs incubated at the same temperature (Table 1). The incubation duration is inversely correlated with the embryo stage at egg-laying for the whole dataset (n = 22, rs = -0.89, $P < 0.01$), for the French sample (n = 11, rs = -0.92,

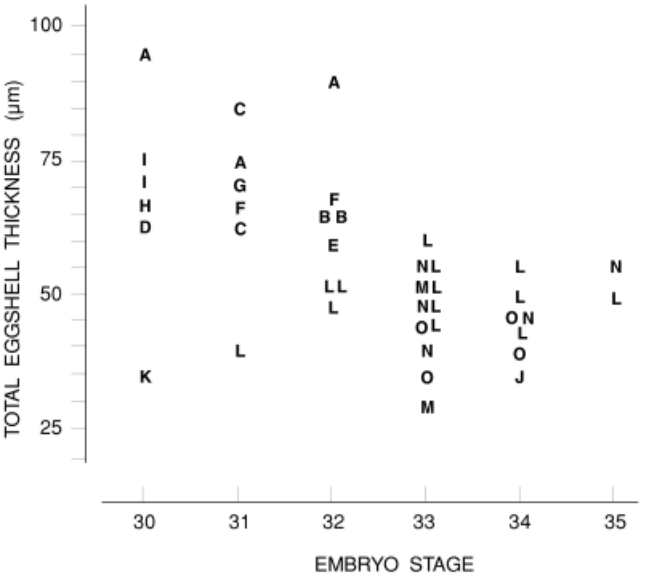

Fig. 2. Scattergram of eggshell thickness vs. embryo stage at egg-laying. Eggs from the Sloveno-Italian populations (**A**: Kot; **B**: Zelenci; **C**: Cerknisko; **D**: Ig; **E:** Tarvisio; **F:** Fusine; **G:** Oropa; **H:** Lac majeur; **I:** Varese) and from the French populations **(J:** Col de Port; **K:** Palomières; **L:** Louvie; **M:** Benou; **N:** Gabas; **O:** Pourtalet).

$P < 0.01$) and for the Sloveno-Italian sample (n = 11, rs = -0.74, $P < 0.01$).

DISCUSSION

The eggshell structure observed in *Lacerta vivipara* is a well-developed fibrous layer overlain by a relatively thin crystalline crust. The EDS analysis indicated that the crust is mainly calcium carbonate. We have previously shown by X-ray diffraction analysis that the calcium carbonate is in the form of calcite (Heulin, 1990). These characteristics correspond to the typical parchment-shelled eggs observed in most (except two subfamilies of Gekkonidae) squamates (Packard et al., 1982; Schleich and Kastle, 1988; Packard and Demarco, 1991).

The two oviparous groups of *Lacerta vivipara*, which belong to two distinct clades, are also reproductively distinct; the eggs laid by females of the Sloveno-Italian populations have thicker eggshells, contain embryos on average less developed at the time of oviposition, and require longer incubation period before hatching than the eggs laid by females of the French populations. Thus, these two oviparous clades might represent an earlier and a later stage, respectively, on an oviparity–viviparity continuum. This comparison of two oviparous groups as well as our previous comparisons of oviparous / F1 hybrids / viviparous samples (Heulin et al., 1992; Arrayago et al., 1996; see introduction) supports the idea that an increase in the developmental stage reached by the embryo at oviposition is associated with a decrease in the eggshell thickness in *L. vivipara*. However, it may be noted that this inverse relation between eggshell thickness and the embryo stage at egg-laying, although supported by our pairwise comparison of the Sloveno-Italian/French clades and by a correlation test performed on the whole dataset, is not verified within each of the clades considered. This may be due either to our relatively small sample sizes or to the influence of additional factors that were not considered in this study (e.g., see below, the remark about the possible role of the vascularization of the extraembryonic membranes and of the oviducts).

There are few models for which it is possible to compare both the embryonic stage at oviposition and eggshell thickness between conspecific populations. In the oviparous lizard *Sceloporus scalaris*, females from a medium-altitude population lay eggs with an eggshell thickness of 27 μm that contain embryos at stage 33, whereas females from a high-elevation population lay eggs with an average eggshell thickness of 19 μm that contain embryos at stage 36 (Mathies and Andrews, 1995). The Australian lizard *Saiphos equalis* is reproductively bimodal. Oviparous females from near Sydney oviposit eggs that contain embryos at an advanced stage of development (around stage 39), whereas viviparous females from the Ryamuka region lay fully formed newborns (i.e., at the final embryonic stage 40) (Smith and Shine, 1997). In this species, the eggshell of the oviparous form is slightly calcified and is 9 μm thick, whereas the eggshell membrane enveloping the embryo of the viviparous form is uncalcified and has a thickness of 5 μm (Smith, unpublished data, pers. commun.). Another Australian lizard, *Lerista bougainvilli*, has three groups of populations that are reproductively distinct: an oviparous group (from southwestern and south-central Australia) in which freshly laid eggs contain embryos at stage 33 and have a 23-μm thick eggshell; another oviparous group (from southeastern Australia) in which freshly laid eggs contain embryos at stage 36 and have a 19-μm thick eggshell; and a viviparous group (from Tasmania and Kangaroo Island) in which the embryo remains encased in 6-μm thick eggshell membrane up to parturition (Qualls, 1996). All these examples (including ours on *Lacerta vivipara*) are consistent with the hypothesis that an increase in egg retention period may be associated with a decrease in eggshell thickness along the oviparity–viviparity continuum. As briefly explained in the introduction, two nonexclusive hypotheses account for the fact that the eggshell of viviparous species is much more reduced (even absent for some species) than those of oviparous species of squamates. It is worth examining here the extent to which these hypotheses could account for variations in eggshell thickness among closely related oviparous taxa.

The first hypothesis considers that gas diffusion is slower through a liquid (i.e., in the oviduct) than in the air (i.e., in the nest). This hypothesis posits that a decrease in eggshell thickness facilitates maternal–fetal gas exchange and, hence, accommodates the increase in the demands for gas exchange when embryos continue their development in utero for longer periods (Packard et al., 1977; Shine and Bull, 1979; Xavier and Gavaud, 1986; Qualls, 1996). It is, however, worth noting that females of several species of sceloporine lizards are able to respond to drought (i.e., an unfavorable condition for nesting) by retaining their eggs in utero past the time of normal oviposition, and that the ability of their embryos to develop further during this prolonged retention does not depend on the eggshell thickness. This observation suggests that a decrease in eggshell thickness is only one of the possible mechanisms that may allow the embryo to avoid hypoxia and hence to continue its development during a prolonged retention in the oviducts (Andrews and Mathies, 2000). Indeed, the timing of development and the level of vascularization of the extraembryonic membranes (yolk sac and chorioallantois) and of the oviduct may play an important role in fetal–maternal gas exchange and may also account for differences in the capacity to support embryogenesis in utero among squamate taxa (Guillette and Jones, 1985; Andrews, 1997).

A second hypothesis, which is an extension of the first, posits that a decrease in eggshell thickness may also accelerate the diffusion of embryonic chemicals signals toward the mother, and that the increased level of those embryonic signals in the target tissues of the mother could then activate endocrinological processes (e.g., stimulation of progesterone secretion by corpora lutea, inhibition of prostaglandin secretion in the oviducts), delaying the time of oviposition (Guillette, 1991, 1993). This idea, which has not yet received much attention in reptiles, might, however, account for a decrease in eggshell thickness along the oviparity–viviparity continuum in squamates; the thinner the eggshell, the faster the diffusion of the embryonic signals into the mother's tissues, the longer the oviposition time is delayed. To date, only two studies have addressed this problem, one suggesting that the embryo of viviparous *Lacerta vivipara* releases chemical signals influencing the life span of the corpora lutea (Xavier et al., 1989) and one indicating that the egg of *Anolis pulchellus* influences histological changes of the oviduct (Ortiz and Morales, 1974). The chemical nature of those embryonic signals in squamates is not known and their possible influence on the length of egg retention remains to be demonstrated.

In conclusion, there are a few examples (including our own on *Lacerta vivipara*) suggesting that some lineages of oviparous squamates may have undergone an evolutionary process involving both a decrease in eggshell thickness and an increase in the period of intrauterine retention of eggs. Viviparous species of squamates, whose eggshells are always strongly reduced or even absent, may represent ultimate steps of this evolutionary process. One may, however, suspect that a decrease in eggshell thickness, although certainly important, is not the only mechanism available to overcome the new physiological constraints (i.e., the problem of gas exchange) associated with a prolonged intrauterine retention of eggs in squamates. In particular, one can wonder whether, depending on lineages, a further vascularization of the extraembryonic membranes and of the oviduct may have preceded, occurred concomitantly with, or followed the decrease in eggshell thickness and the increase of the period of intrauterine retention of eggs. Future studies should also not neglect the hypothesis that the length of intrauterine retention might directly depend on the intensity of a maternal–fetal chemical communication through the eggshell barrier

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