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Identification of a contact zone between oviparous and viviparous common lizards (*Zootoca vivipara*) in central Europe: reproductive strategies and natural hybridization

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Abstract. The European common lizard, *Zootoca vivipara*, is one of the very few reptile species with two reproductive modes, viz. viviparity and oviparity. Oviparity in this otherwise viviparous form has been known since 1927 for the allopatric *Z. v. louislantzi*. Only with the discovery of a second oviparous form, *Z. v. carniolica*, a parapatric occurrence of oviparous and viviparous populations became conceivable. In this study, we (1) detect a contact zone where both forms meet, (2) find evidence for natural hybridization between both reproductive strains, and (3) compare the reproductive strategies of egg-layers and live-bearers independent from environmental interference. Thirty-seven gravid females were captured in a supposed contact zone in Carinthia, Austria, and maintained in the laboratory until oviposition or parturition. Clutch size, embryonic mortality and birth weight of the neonates were compared among the reproductively differentiated samples. Hybrids were identified by intermediate reproductive characteristics. Our results provide the first proof of a contact zone between live-bearing and egg-laying *Z. vivipara*, and of natural hybridization among them. The study further provided evidence that hybrid females of the two strains are at least partially fertile, and that oviparous and viviparous *Z. vivipara* show clear differences in their reproductive strategies.

Key words. Squamata, Lacertidae, Austria, birth weight, clutch size, eggshell, oviparity, reproductive mode, viviparity.

Introduction

Within squamate reptiles, viviparity (live-bearing) has evolved from oviparity (egg-laying) independently more than 100 times, which is more frequently than in all other vertebrates combined. The transition evolved often at low taxonomic levels and in geologically recent times (Blackburn 2005). Therefore, squamate reptiles are ideal for studies on the evolution of viviparity.

Several hypotheses have been developed to explain the high number of transitions to viviparity in squamate reptiles. The most generally applicable and widely accepted theory is the cold climate hypothesis (Tinkle & Gibbons 1977, Shine 1985). According to this theory, the advantages of viviparity are that the retention of embryos in the uterus accelerates embryonic development in cold climates because the temperature inside the uterus can be warmer than that of the soil. The faster development leads to an earlier birth, which may be adaptive in cold climates because it is more likely that juveniles are born prior to the onset of the first frosts. Likewise, more time remains to accumulate energy reserves before hibernation, which may be crucial for the survival of the offspring until next spring. Furthermore, embryos inside the uterus are protected from some environmental sources of mortality like egg predation and egg dehydration. However, there are disadvantages as well. Although viviparity may increase the survivorship of the offspring, it is presumably more costly to the reproducing female. Reduced mobility during pregnancy may increase the vulnerability to predation and/or decrease the feeding ability of the female and thus diminish subsequent fertility. The long period of egg-retention impedes live-bearers to produce several clutches per year, which potentially leads to further reduction in fertility compared to their egg-laying sister taxa. Thus, although viviparous offspring might survive better in cold climates, egg-layers potentially show a higher fertility in mild climatic conditions. Support for the cold climate hypothesis comes from the fact that in squamate reptiles, the viviparous mode of reproduction is proportionately more common at higher latitudes and with increasing elevation (for a review see Shine 1985; but see also Andrews 2000, Shine et al. 2003, Shine 2004).

In viviparous reptiles, provision of nutrients to the embryo via complex placental tissues (‘placentotrophic viviparity’) evolved only a few times. Rather, most viviparous squamates show a pattern that can be viewed as ‘extreme egg-retention’, where nutrients are still provided by yolk. Although incipient placentae exist in this case as well, they are mainly responsible for gas exchange and water supply (Blackburn 2005). Following the terminology of Blackburn (1994), this form of viviparity can be termed 'lecithotrophic viviparity' (live-bearing and mainly vitelline nutrients).

Only a few reproductive bimodal reptile species are known, making a phylogenetically undistorted comparison between oviparous and viviparous forms difficult. One of these very few species is the European common lizard *Zootoca vivipara* (Jacquin, 1787). Lecithotrophic viviparous forms of this species (for simplicity, we will use the term 'viviparous' from here onwards) are widely distributed from Central France and the British Isles to the North Cape in Scandinavia and north-eastern Asia as far as Japan (Dely & Böhme 1984), whereas oviparous populations are restricted to some southern margins of its range. Currently, two different oviparous forms are known, which were recently described as the subspecies *Z. vivipara carniolica* Mayer, Böhme, Tiedemann & Bischoff, 2000 and *Z. v. louislantzi* Arribas, 2009. *Zootoca v. louislantzi* occurs in Southwestern Europe in the Pyrenees, Aquitaine and the Cantabrian Mountains, geographically isolated from the viviparous forms (Lantz 1927, Braña & Bea 1987, Heulin 1988, Arribas 2009). Thus, hybridization between *Z. v. louislantzi* and the viviparous forms cannot occur in nature, even though experimental hybridization demonstrated that they are capable of it (Heulin et al. 1992, Arrayago et al. 1996). Heulin et al. (1992) and Arrayago et al. (1996) found out that F_1 hybrid females (oviparous × viviparous and vice versa) laid eggs with intermediate characteristics between regular oviparous and viviparous reproduction. Compared to usual oviparous eggs, F_1 hybrid eggs possessed an only partially calcified and thinned shell, were laid at an advanced stage of embryonic development and had a shortened incubation period. Although the global values of breeding success of F_1 hybrids tended to be lower than that of normal oviparous or viviparous reproduction, it seemed that most, if not all, crosses including F_1 hybrids were potentially viable (Heulin et al. 1992, Arrayago et al. 1996).

With the discovery of a second strain of oviparous common lizards in south-eastern Europe, namely *Z. v. carniolica* (Mayer et al. 2000), detection and subsequent investigation of a natural hybrid zone between oviparous and viviparous forms became conceivable. *Zootoca v. carniolica* occurs in Slovenia, northern Italy, northwest Croatia and the Austrian Carnian Alps. Although an encounter with viviparous forms is expected (Mayer et al. 2000, Heulin et al. 2000, Surget-Groba et al. 2002), no contact zone has been identified so far. It is therefore unknown whether natural hybrids exist or not, nor how a possible hybrid zone might be structured.

Studies on the phylogenetic relationships among oviparous and viviparous *Z. vivipara* using karyotype, chromosome structure and mitochondrial DNA polymorphisms revealed that the south-eastern oviparous strain, *Z. v. carniolica*, forms a basal lineage. It is clearly distinct from the south-western oviparous lineage, *Z. v. louislantzi*, which is more closely related to the viviparous clades. Moreover, it turned out that neither the viviparous nor the oviparous forms are monophyletic. This circumstance also induced a debate whether viviparity arose more than once in different populations of the species or a single origin of viviparity was followed by a reversal to oviparity (Mayer et al. 2000, Surget-Groba et al. 2001, 2006, Odierna et al. 2001, 2004, Kupriyanova et al. 2006).

A comparison between the two oviparous lineages also revealed differences in some reproductive characteristics such as eggshell thickness (*Z. v. carniolica*: 72.0 µm, range 62–94 µm; *Z. v. louislantzi*: 46.3 µm, range 28–62 µm) and embryonic developmental stage at oviposition (*Z. v. carniolica*: 31, range 30–32; *Z. v. louislantzi*: 33, range 30–35; stages according to Dufaure & Hubert 1961) (Heulin et al. 2002).

Owing to these differences in their reproductive characteristics and their phylogenetic distinctiveness, the outcomes of a hybridization experiment between *Z. v. carniolica* and viviparous common lizards might differ from the findings of the crossbreed between *Z. v. louislantzi* and live-bearing strains of *Z. vivipara*. Moreover, habitat differences, mating choice or selection against hybrids may minimize the occurrence of hybrids under natural conditions.

As no contact zone between oviparous and viviparous *Z. vivipara* has been identified so far, it is unknown how strong the selective advantages for one particular reproductive mode might be. The superior form might displace the inferior strain completely or until a geographic barrier is reached. For example, it has been considered whether allopatry was a necessary condition for the survival of the oviparous *Z. v. louislantzi* (Surget-Groba et al. 2002). Furthermore, it is conceivable that hybridization between the two reproductive modes might break up the linkage between involved adaptive traits, and leads to strong selective disadvantages of genetically intermingled forms compared to "pure" oviparous or viviparous lineages.

The aims of this study were therefore to (1) detect a possible contact zone between oviparous *Z. v. carniolica* and viviparous *Z. vivipara*, (2) find evidence for natural hybridization between these two forms and examine whether possible hybrids are fertile or not, and (3) compare the reproductive strategies between egg-layers and live-bearers relatively free from environmental and phylogenetic interference.

Materials and methods

Sampling

Thirty-seven gravid females of *Z. vivipara* were collected in Carinthia, Austria (46°36.20' – 46°35.64' N, 13°07.84' – 13°08.51' E, 1368–1575 m a.s.l.), in a supposed contact zone between oviparous *Z. v. carniolica* and viviparous *Z. v. vivipara*. Presence of a mating bite scar on the belly of the female was used to identify gravidity. Lizards were reared separately or, if easy to distinguish from each other, two by two in plastic terraria (30 × 20 × 20 cm). Each terrarium was equipped with some soil, moss, a shelter and a water bowl. The lizards were fed with spiders, small grasshoppers and house crickets. A 15 W terrarium lamp provided 23–28°C for 8 h/day. Lizards were checked daily for lateral skin folds which occur as a result of oviposition or parturition, respectively. In the case of skin folds having appeared, the terrarium was browsed for eggs or neonates, which were subsequently separated from the females. Eggs were incubated in vermiculite at 23–24°C using a Bruja 3000/REP incubator. Due to low external temperatures, two clutches (laid by females VK-125 and VK-089) were incubated at 21–22°C for the first 26 and 9 days, respectively. Females and their offspring were released after the experiment at the exact capture localities of the mothers.

Embryonic development and scanning electron microscopy of eggshells

One egg was taken from each clutch immediately after oviposition, preserved in 70% ethanol, and dissected afterwards in order to determine the developmental stage of the embryo according to Dufaure & Hubert (1961). If embryonic development fell between two stages, the arithmetic mean was used for further calculations. Additionally, scanning electron microscopy (SEM) analyses were performed for six eggshells (eggs laid by females VK-89, VK-90, VK-122, VK-128, VK-143 and VK-145). Eggshells were cleaned from yolk remnants, stored in 96% ethanol overnight, and air-dried afterwards. In order to prevent shrinkage during drying, the eggshells were placed over the rounded end of a wooden holder. Dried eggshells were cut into 2–10 mm² fragments and affixed onto specimen stubs with double-sided carbon tape. At most one millimetre-wide eggshell strips were used for measuring thickness. Samples were gold-coated (40 nm) with an Anatech Hummer VII Sputtering System and examined with a Hitachi S-2460N SEM at 25 kV. Images of the eggshell surface were taken at × 200 and × 1250 magnification. Thickness of the entire eggshell, including calcareous layer, fibres of shell membrane and inner boundary (terminology according to Packard et al. 1982) was determined by averaging measurements at five different sites of the eggshell, using a suitable magnification for each sample.

Measurements, calculations and statistics

The ratio $N_d / (N_d + N_a)$ was used to calculate embryo mortality, with N_a being the number of hatched or born neonates alive and N_d the number of sterile eggs, embryos with ceased development or stillborn offspring. For viviparous females, big yolk lumps extruded at parturition were assumed to belong to remains of necrotic embryos and were therefore counted as dead embryos. Using this equation, eggs removed for the examinations mentioned above were not taken into account. Fertility was calculated by subtracting N_d from the entire clutch or litter size in order to judge eggs that were removed for the examinations mentioned above as potentially viable. Neonates were weighed with precision scales Mettler 2500 Delta Range after hatching or birth, respectively. Birth weight was averaged per clutch / per litter in order to prevent dependence

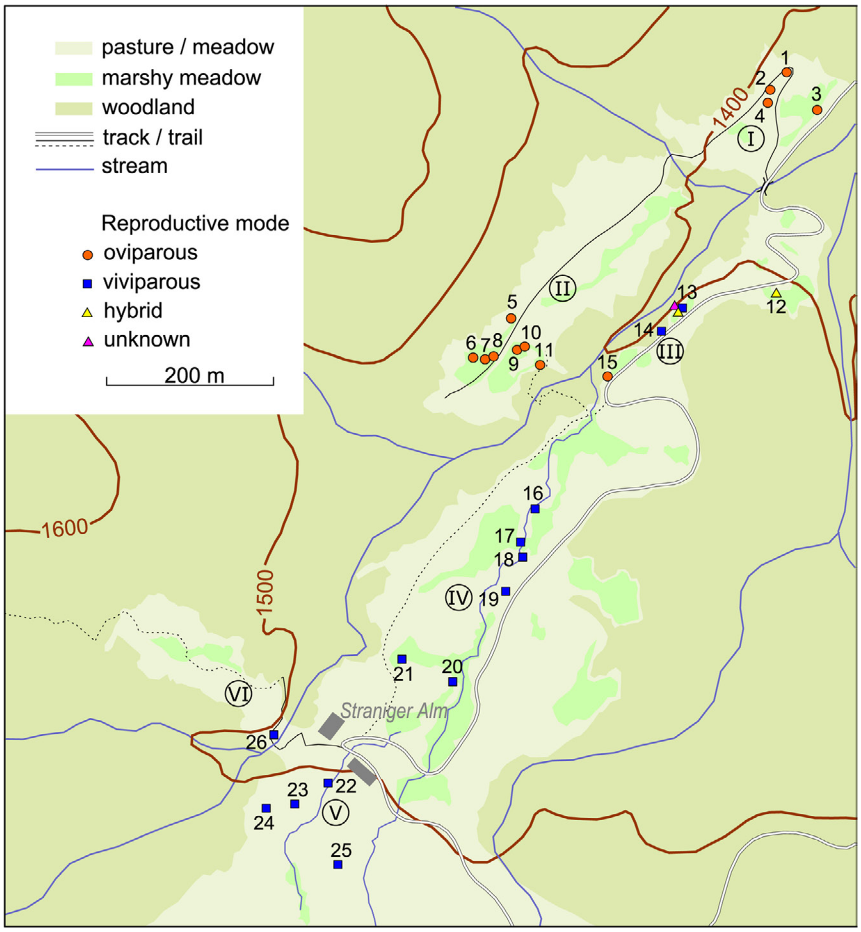

Figure 1. Localities of oviparous, viviparous and hybrid females of *Z. vivipara* at the study site 'Straniger Alm'. Detailed information is given in App. 1.

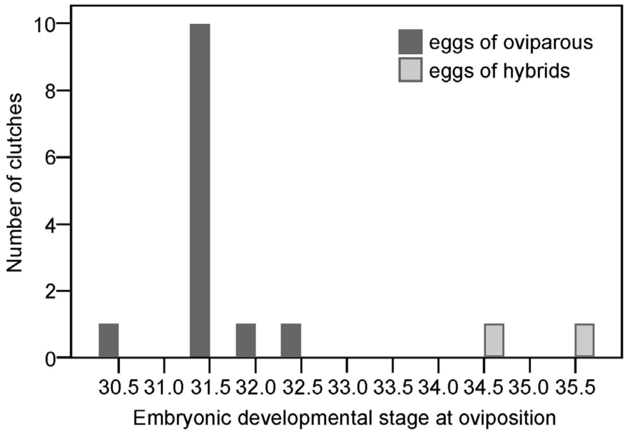

Figure 2. Embryonic developmental stages at oviposition (Dufaure & Hubert 1961) for oviparous and hybrid clutches of *Z. vivipara*.

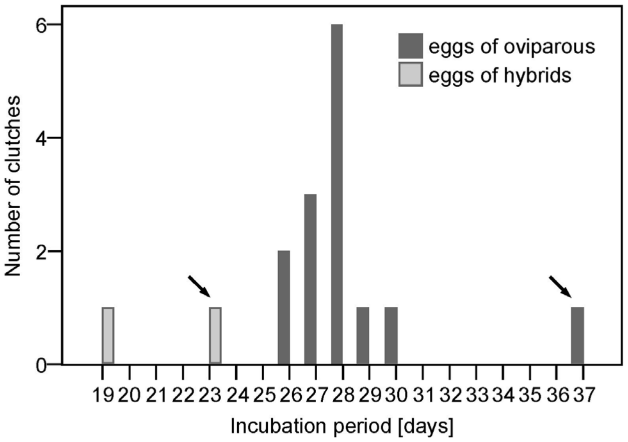

Figure 3. Incubation periods for oviparous and hybrid clutches of *Z. vivipara*. Incubation temperature: 23–24°C. Arrows indicate two clutches that were incubated at 21–22°C for the first 9 and 26 days, respectively.

effects between individual values. Snout–vent length (SVL) was measured for each adult female. Incubation period indicates the days from oviposition to hatching of the first juvenile of each clutch.

Statistical analyses were performed with SPSS 14.0 for Windows. Data were checked for normal distribution (Kolmogorov-Smirnov-test) and homogeneity of variance (Levene-test). For data that fulfilled the requirements for parametric methods, a *t*-test for independent samples or a one-way ANOVA was used; otherwise a Mann-Whitney-U-test or a Kruskal-Wallis-H-test was applied. For normally distributed data, Pearson's correlation coefficient was calculated; alternatively Spearman's rank correlation coefficient was used. A univariate analysis of variance was performed for the variables "clutch size", "fertility" and "birth weight". A 5% significance level was used for all tests. Hybrids were excluded from statistical tests due to their small sample size.

Results

Study area and capture localities of the 37 lizards examined in this study are depicted in Fig. 1 (for detailed information see App. 1). Fourteen lizards showed a regular oviparous reproduction and were found in sub-areas I, II and III (below 1460 m a.s.l.). Twenty lizards possessed a regular viviparous reproduction and were caught in sub-areas III, IV, V and VI (above 1415 m a.s.l.). Hybrids (samples VK-89 and VK-128) as well as one specimen with unknown reproductive mode (infertile female, sample VK-90) were restricted to sub-area III.

Hybrids between oviparous and viviparous strains were identified according to their intermediate reproductive characteristics comparable to the findings of the hybridization experiments of Arrayago et al. (1996) and Heulin et al. (1992) (see below). One infertile female laid shelled eggs that all turned mouldy a few days after oviposition. The ethanol-preserved egg of this clutch contained no visible embryo and the SEM examination revealed that the surface of the eggshell was anomalous in its structure (see below).

The embryonic developmental stages at oviposition were on average 31.5 (range 30.5–32.5) for the oviparous, but 34.5 and 35.5 for the respective hybrid clutches (Fig. 2). Correspondingly, the incubation period was shortened for the hybrid eggs (Fig. 3, Tab. 1).

The eggshells of the regular oviparous samples were with 70.4, 78.0 and 78.3 µm much thicker than the hybrid ones, which showed a thickness of only 22.0 and 28.8 µm, respectively. These findings coincide well with values already published for *Z. v. carniolica* from Slovenia (72.0 µm; Heulin et al. 2002) and laboratory-reared hybrids (21 µm; Heulin et al. 1992). SEM images of the outer surface of the eggshells revealed an inconsistent structure within samples of the same reproductive mode, but showed clear differences among oviparous and hybrid samples (Figs. 4 and 5). Regular oviparous eggs possessed a surface divided into a large number of polygonal, convex structures that are partitioned from each other by furrows. Most of these polygons are covered with angular calcite crystals of variable sizes that are part of the calcareous layer (Fig. 4 A, B, C, E). In some areas, the large angular crystals are replaced by small granular ones (Fig. 4 D), but in others the calcareous layer is completely absent (Fig. 4 F). In this case the tightly interwoven fibres of the outer surface of the shell membrane, otherwise covered by the calcareous layer, become visible. In contrast to the regular oviparous eggs, the surface of hybrid eggshells is less textured and the conspicuous polygonal structures are not developed (Fig. 5 A–D). Although angular calcite crystals are present and comparable in size to those of regular oviparous eggshells, they are directly arranged on the less tightly interwoven shell membrane instead of being placed on top of the polygonal structures. The shell membrane becomes visible between the individual crystals as well as in some areas where the calcareous layer is completely absent (Fig. 5 D). Figures 5 E and F show the outer surface of an eggshell with an apparently anomalous structure. It was laid by one female whose complete clutch failed to develop

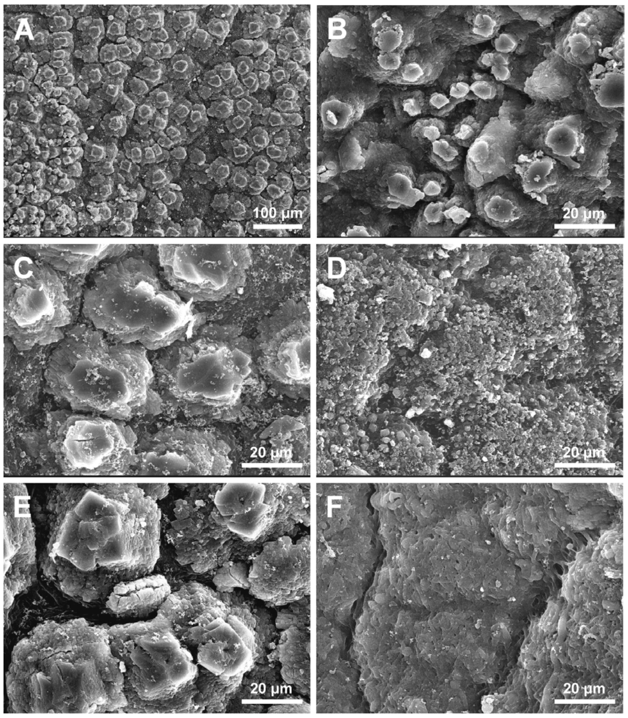

Figure 4. Outer surface of regular eggs of *Z. vivipara*. See main text for details. Scale bars: A, 100 µm; B–F, 20 µm.

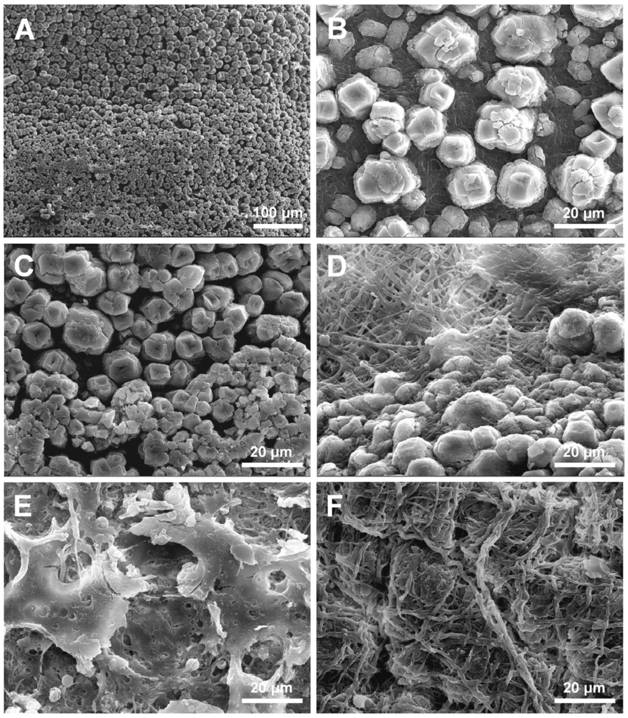

Figure 5. Outer surface of hybrid eggs (A–D) and an eggshell with anomalous structure (E, F) of *Z. vivipara*. See main text for details. Scale bars: A, 100 µm; B–F, 20 µm.

Table 1. Embryonic developmental stages at oviposition and incubation periods of oviparous, viviparous and hybrid clutches of *Z. vivipara*. Developmental stages according to Dufaure & Hubert (1961). Incubation temperature: 23–24°C; two clutches, one of an oviparous and one of a hybrid female, were incubated at 21–22°C for the first 26 and 9 days, respectively.

Variable	Reproductive mode	N	Median	IQR	(Range)
Developmental stage	oviparous	13	31.5	0.00	(30.5 – 32.5)
	hybrid	2	35.0	–	(34.5 – 35.5)
Incubation period [days]	oviparous	14	28	1.25	(26 – 37)
	viviparous	20	0	1.75	(0 – 6)
	hybrid	2	21	4.00	(19 – 23)

(VK-90). Angular calcite crystals are lacking; in fact the surface consists of a compact but perforated crust (Fig. 5 E) and tightly interwoven fibres (Fig. 5 F). With a thickness of 41.5 µm this eggshell clearly exceeds the range of the hybrid samples.

Based on the properties of their clutches (advanced embryonic development, shortened incubation period and reduced eggshell thickness), samples VK-89 and VK-128 were identified to be natural hybrids between oviparous and viviparous *Z. vivipara*. The reproductive mode of the infertile sample VK-90 remains unknown; hence this specimen was excluded from further analyses.

Snout-vent length (SVL) of the reproductive females was significantly larger for viviparous lizards (p = 0.010, *t*-test). Live-bearers also produced larger litters, but this was only marginally significant (p = 0.050, *t*-test) (Tab. 2). SVL and litter size showed a statistically significant positive linear relationship for viviparous lizards (r = 0.522, p = 0.018, N = 20; Pearson's correlation) but not for oviparous ones (r = 0.318, p = 0.268, N = 14; Pearson's correlation) (Fig. 6). For pooled data sets, the correlation was highly significant (r = 0.544, p = 0.001, N = 34). A univariate analysis of variance (dependent variable: litter size; factor: reproductive mode; covariate: SVL) revealed significant relationships between litter size and SVL (p = 0.006) independent of reproductive mode (p = 0.455).

There was no significant difference in embryo mortality between both reproductive modes (p = 0.796, Mann-Whitney-U-test) (Tab. 3). Fertility was higher for viviparous females, but this was not significant (p = 0.060, *t*-test) (Tab. 2). Instead, a univariate analysis of variance revealed that the differences in fertility could be ascribed to SVL (p = 0.003 for SVL, p = 0.549 for reproductive mode).

Birth weight was significantly higher for oviparous lizards (p < 0.001, *t*-test) (Tab. 2, Fig. 7), not correlated with clutch size (Pearson's correlation; oviparous: r = 0.066, p = 0.829, N = 13; viviparous: r = 0.130, p = 0.645, N = 15), and a univariate analysis clearly confirmed that birth weight depends on reproductive mode (p = 0.667 for clutch size, p = 0.069 for SVL, p < 0.001 for reproductive mode).

Due to their small sample size, it was not possible to statistically compare the reproductive characteristics of hybrids with those of their parental taxa. Nevertheless, for the few data available, it seems that embryo mortality was slightly higher in hybrids albeit this was compensated by their large clutch sizes. As a result, fertility was comparable to the oviparous samples. Birth weights of hybrid offspring were intermediate between oviparous and viviparous neonates (Tab. 2, Fig. 7).

Discussion

Evidence of natural hybrids

This study provides the first evidence of natural hybridization between oviparous and viviparous strains of *Z. vivipara*. According to the properties of experimentally produced hybrid clutches described by Arrayago et al. (1996) and Heulin et al. (1992), two females could be unambiguously identified to be hybrids due to the advanced development of their embryos at oviposition, the shortened incubation period of their eggs, and the reduced eggshell thickness. Developmental stages of 34.5 and 35.5 were similar to 35–36 recorded by Arrayago et al. (1996), and the eggshells were with 22.0 and 28.8 µm only slightly thicker than the average of 21 µm described by Heulin et al. (1992). Incubation period was not compared with literature values as it is negatively correlated with incubation temperature (Heulin et al. 1991, Osenegg 1995, Arrayago et al. 1996). The slight deviations between the reproductive traits of the experimental hybrid strain (viviparous × *Z. v. louislantzi*) and the natural hybrids (viviparous × *Z. v. carniolica*) probably refer to the differentiation between *Z. v. louislantzi* and *Z. v. carniolica* in terms of developmental stages at oviposition and eggshell characteristics (Heulin et al. 2002), possibly due to the genetic differentiation between the two oviparous clades (Mayer et al. 2000, Odierna et al. 2004, Surget-Groba et al. 2001, 2006). Certainly, more data are needed for a proper comparison. Since it is possible that the reproductive mode acts like a Mendelian character (Arrayago et al. 1996), no conclusion can be drawn on whether the two females found in the contact zone are F_1 or F_n hybrids, or backcross progeny. Nonetheless, it is noteworthy that the two hybrid females produced viable offspring, even though the further fate of the progeny could not be observed because they were released after the experiment.

With a thickness of 41.5 µm, the eggshell generated by the infertile female was similar to those of *Z. v. louislantzi* (28–62 µm), but falls below the lower limit of *Z. v. carniolica* (62–94 µm) and seems to exceed the value of the hybrids (average 21 µm) (Heulin et al. 1992, 2002). Moreover, SEM examinations revealed an anomalous structure compared to regular oviparous or hybrid eggshells. As eggshell formation occurs rapidly during early embryo development and varieties in thickness result from uterine glandular layer differences rather than subsequent eggshell degeneration (Heulin et al. 2005), it is possible that the infertile female was a regular oviparous one. Infertility might be caused by a dysfunction in shell segregation, which led

Table 2. Reproductive characteristics and SVL of oviparous, viviparous and hybrid females of *Z. vivipara*. Birth weight averaged per clutch / per litter.

Variable	Reproductive mode	N	Mean	± SD	(Range)	p value (*t*-test)
SVL [mm]	oviparous	14	60.6	± 3.46	(55 – 68)	0.010
	viviparous	20	64.5	± 4.53	(58 – 76)	*
	hybrid	2	62.0	± 0.00	(62 – 62)	
Clutch / litter size	oviparous	14	5.4	± 1.09	(4 – 7)	0.050
	viviparous	20	6.5	± 1.64	(3 – 10)	
	hybrid	2	7.5	± 0.71	(7 – 8)	
Fertility	oviparous	14	4.8	± 1.37	(2 – 7)	0.060
	viviparous	20	5.8	± 1.58	(2 – 9)	
	hybrid	2	5.0	± 1.41	(4 – 6)	
Birth weight neonates [mg]	oviparous	13	277.2	± 42.05	(183 – 346)	0.000
	viviparous	15	201.0	± 19.33	(163 – 229)	***
	hybrid	2	226.2	± 0.49	(226 – 227)	

Table 3. Embryo mortality for oviparous, viviparous and hybrid clutches of *Z. vivipara*.

Variable	Reproductive mode	N	Median	IQR	(Range)	p value (U-test)
Embryo mortality	oviparous	14	0.000	0.288	(0.00 – 0.67)	0.796
	viviparous	20	0.000	0.161	(0.00 – 0.50)	
	hybrid	2	0.393	0.214	(0.29 – 0.50)	

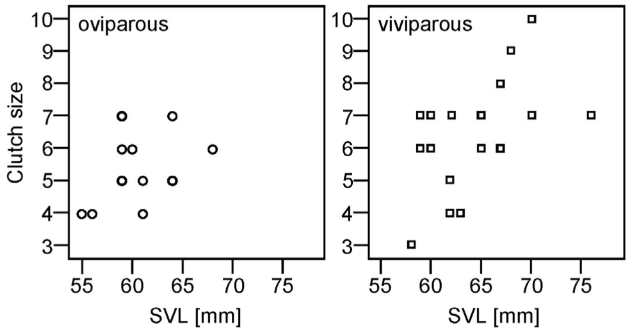

Figure 6. Clutch / litter size plotted against SVL for oviparous and viviparous *Z. vivipara*. Number of samples: oviparous, 14; viviparous, 20.

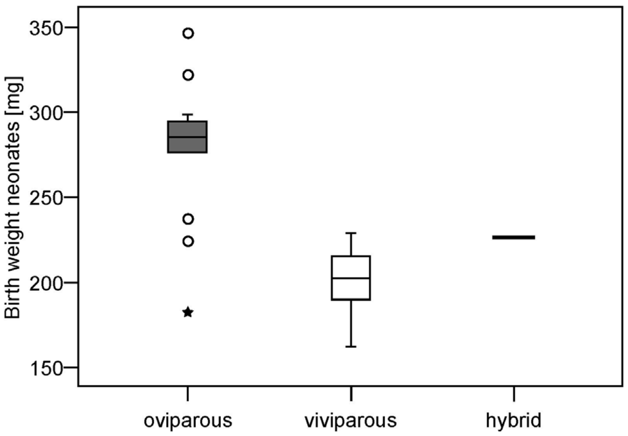

Figure 7. Birth weights of oviparous, viviparous and hybrid offspring (averaged per clutch / per litter) of *Z. vivipara*. Number of samples: oviparous, 13; viviparous, 15; hybrids, 2.

to an early demise of the embryos. On the other hand, this female was captured in the middle of the contact zone in close proximity to the hybrids, thus it is also conceivable that this sample was an infertile hybrid. Genetic analyses are needed to clarify this issue.

Eggshell characteristics

The eggshell characteristics of the regular oviparous samples were very similar in thickness and surface structure to former findings on *Z. v. carniolica*. For instance, the eggshell thicknesses of 70.4, 78.0 and 78.3 µm of the current study coincide well with the 62–94 µm noted for a Slovene-Italian sample (Heulin et al. 2002) that corresponds to *Z. v. carniolica*. However, to our knowledge, the absence of angular crystals in some parts of the eggshell has not been reported yet (Heulin 1990, Heulin et al. 1992, 2002). These areas resemble roughly those of some other oviparous lizards like *Anolis limifrons* (Sexton et al. 1979) and some structures in *Callisaurus draconoides* eggs (Packard et al. 1982). The uncalcified patches of the hybrid eggs, by contrast, look completely different from these zones.

The hybrid eggshells of the current study are more consistently covered with calcium crystals than those from the (viviparous × *Z. v. louislantzi*) hybrid strain. A heterogeneous appearance of the eggshells with opaque and translucent areas due to patchy calcification (as described by Arrayago et al. 1996 and Heulin et al. 1992) could not be observed. These differences in eggshell characteristics might be due to the distinct phylogenetic background of the taxa involved in the different crosses.

Differences in reproductive strategies

This study provided a scarce opportunity to acquire data from directly adjacent populations of two closely related taxa with different modes of reproduction. This allowed comparing oviparous with viviparous reproductive strategies relatively free from phylogenetic and environmental interferences. The most striking differences between the two populations were found in the birth weight of neonates, clutch size, and SVL of females.

Birth weights of oviparous hatchlings were on average 1.4 times higher than those of viviparous neonates and showed an intermediate expression in the hybrids. Hence it seems likely that this trait is linked to the genotype of the mother, for instance through differences in hormonal regulation, oxygen availability or yolk supply. For example, it has been shown for viviparous *Z. vivipara* that most females produce smaller offspring than they would be physically capable of (Olsson et al. 2002). Lighter neonates are likely to reduce the burdens for the pregnant female and therefore increase productivity for the next or the ongoing season. However, at a high altitude, activity periods are short. Juveniles have little time to feed and accumulate energy reserves before hibernation, thus a higher weight at birth would enhance their probability of surviving until next spring. In accordance with this presumption, birth weight increases with altitude for *Z. v. louislantzi* (see Osenegg 1995). The investment of the mother in the weight of their progeny seems therefore to be a balancing act, especially for viviparous reproduction where a high embryonic weight increases the burdens for the female. An earlier birth date of the viviparous juveniles, due to their faster development inside the uterus (Shine & Bull 1979, Shine 1985, Heulin et al. 1991), might compensate their disadvantage compared to the heavier oviparous hatchlings in the adjacent population.

No difference was found in embryo mortality under laboratory conditions. But, it is not clearly known if unviable embryos of *Z. vivipara* are extruded at the time of birth or not (Jacobi 1936, Blackburn et al. 2003). Because in the present study some females extruded at parturition big yolk lumps that were markedly larger than the residual yolk of fully developed neonates, it seems probable that these were remains of necrotic embryos. Using these yolk lumps as indication of unviable embryos would allow a comparison of embryo mortality between the reproductive modes. Nevertheless, embryo survival is highly speculative because parameters such as egg predation or vulnerability of pregnant females under natural conditions could not be assessed.

Snout–vent length and clutch size were positively correlated in oviparous as well as viviparous common lizards, although this relation was not significant for the egg-layers, possibly due to their small sample size. A univariate analysis of variance revealed that the relation between clutch size and SVL is not influenced by reproductive mode. The strong correlation between SVL and clutch size is known for *Z. vivipara* (Peters 1962, Pilorge 1987, Osenegg 1995). Because viviparous reproductive females from the contact zone were significantly larger than oviparous ones, they were capable of producing more offspring, supposing that only one clutch per year is generated. Although this seems likely for the high altitude of the study site, it cannot be readily inferred because multiple clutches are frequently laid by lowland populations of *Z. v. louislantzi* (Osenegg 1995, Heulin et al. 1991) and have also been reported for *Z. v. carniolica* even at an altitude of 1040 m (Heulin et al. 2000).

As clutch size is an important reproductive trait, it would be interesting to learn why viviparous females have a significantly larger SVL than oviparous females of the neighbouring population. Possible causes include (1) the reproductive mode itself, (2) altitude, (3) microhabitat, and (4) age structure.

(1) A comprehensive study of female specimens of the genus *Liolaemus* showed a higher axilla-groin / SVL relationship for viviparous than oviparous females, which was explained by larger space requirements for embryonic growth in live-bearing lizards (Cei et al. 2003). Even so, this is not a general rule. *Sceloporus scalaris*, for example, has a smaller SVL at high altitude although eggs are laid with an advanced stage of embryonic development compared to those of lowland populations of the same species (Mathies & Andrews 1995). Even studies on *Z. vivipara* give no clear pictures. Either no differences in body size were found, or viviparous forms showed a smaller SVL than *Z. v. carniolica* (Guillaume et al. 2000, 2006). (2) Altitude could have an influence on body size if Bergmann's rule is true for lizards. This is the subject of controversial discussions (Olalla-Tárraga et al. 2006, Pincheira-Donoso et al. 2008, Angilletta et al. 2004). (3) Microhabitat in terms of prey abundance seems to be a major factor in determining different body size patterns even in nearby populations (Pilorge 1987). Habitat differences were not investigated in the present study, but hillside orientation (Fig. 1) and temperature course (not shown) differed among sub-areas. However, it is hard to tell how much this might influence SVL given the short distances between localities. (4) In colder environments, lizards delay maturation until reaching a larger body size (Angilletta et al. 2004) and an increase in mean age with higher altitude has been reported for various lizard species (Roitberg & Smirina 2006, and references therein).

With the sparse data available for the contact zone, it is impossible to deduce which causes might be responsible for the obvious differences in SVL between oviparous and viviparous females. Although altitude differs only slightly within the investigated area, an influence from adjacent populations is conceivable. Because the analyses showed that clutch size is not influenced by reproductive mode but is positively correlated with SVL, oviparous and viviparous females of the same size produce similar numbers of offspring. Nevertheless, a larger body size would enhance relative uterine expansion ability. That means that if there is an increased uterine space requirement for viviparous *Z. vivipara*, a larger body size due to delayed maturity, for example, would be adaptive by increasing relative uterine expansion ability but would not alter the SVL / clutch size correlation. Thus, a combination of (1) the reproductive mode itself, and (4) age structure, seems conceivable.

Inferences from the contact zone

The main parts of the oviparous and viviparous populations are separated by a small, wooded stream valley (Fig. 1) that forms a shallow barrier to dispersal. Since the habitat of the egg-layers is almost completely bounded by woodland, the best possibility to disperse is across the stream into the viviparous population, whereas the viviparous lizards have more options to spread. This might explain the position of the overlap area on the eastern side of the stream (sub-area III).

Oviparous and viviparous lizards clearly show different reproductive strategies. This becomes obvious in differences in birth weight, female SVL and associated clutch / litter sizes. It appears likely that in the contact zone the advantages and disadvantages of both reproductive modes are level. However, the relatively steep transition from oviparous to viviparous reproduction is surprising and suggests selection against hybrids or a sensitive ecological threshold with strong selection against the invading form. Presumably, this threshold is caused by temperature, which has a strong effect on embryonic development in squamate reptiles and is evidently the primary cause of the evolution of viviparity in reptiles (Shine & Bull 1979, Shine 1983, 1985, 2004, Shine et al. 2003, Mathies & Andrews 1995, Heulin et al. 1991, Andrews 2000). In fact, the hillside orientation of areas I and II is more favourable with regard to temperature than areas IV and V, and hence could be responsible for the observed distribution pattern of the two reproductively distinct forms (Fig. 1). This suggests that oviparous *Z. vivipara* might be superior to viviparous forms as long as temperature conditions support a proper development of eggs in the soil.

In the contact zone, where both forms of reproduction are supposed to approach equilibrium, hybrids should not be disadvantaged due to their intermediate characteristics either. Nevertheless, the thin and only slightly calcified eggshell could be more vulnerable to invertebrate predation or dehydration. The relatively high embryo mortality within the two hybrid clutches could also be a clue to genetic incompatibilities between the two parental taxa.

Conclusion

This study provides the first evidence of natural hybridization between oviparous and viviparous forms of *Z. vivipara*. Hybrids are at least partially fertile, even though the reproductive mode of one infertile (possibly hybrid) female remains unknown. Oviparous and viviparous *Z. vivipara* clearly show different reproductive strategies and apart from occasional hybridization, two distinct modes are maintained. It seems therefore that, once viviparity has evolved, both reproductive modes can coexist in parapatry. The position of the contact zone could be caused by a temperature threshold where both reproductive modes are at equilibrium. Measuring gene flow between the two populations as well as hybridization experiments would be useful to gain insight into the degree of differentiation between *Z. v. carniolica* and viviparous forms of *Z. vivipara*.

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Appendix 1

Female *Z. vivipara* specimens examined in this study. (o) oviparous, (v) viviparous, (h) hybrid, (u) unknown reproductive mode.

Sub-area	Locality	GPS coordinates (N, E, altitude [m])			Specimen
I	1	46°36.177’	13°08.452’	1382	VK-078(o)
	2	46°36.168’	13°08.440’	1392	VK-125(o)
	3	46°36.154’	13°08.481’	1377	VK-109(o)
					VK-138(o)
					VK-162(o)
	4	46°36.159’	13°08.436’	1383	VK-165(o)
II	5	46°36.021’	13°08.199’	1447	VK-157(o)
	6	46°35.997’	13°08.165’	1460	VK-143(o)
	7	46°35.997’	13°08.177’	1453	VK-137(o)
	8	46°35.998’	13°08.184’	1455	VK-140(o)
	9	46°36.002’	13°08.207’	1447	VK-145(o)
	10	46°36.003’	13°08.212’	1450	VK-122(o)
	11	46°35.993’	13°08.227’	1446	VK-152(o)
III	12	46°36.039’	13°08.442’	1407	VK-128(h)
	13	46°36.028’	13°08.353’	1415	VK-089(h)
					VK-090(u)
					VK-130(v)
					VK-141(v)
	14	46°36.015’	13°08.337’	1419	VK-142(v)
					VK-144(v)
	15	46°35.985’	13°08.288’	1432	VK-035(o)
IV	16	46°35.903’	13°08.221’	1446	VK-149(v)
	17	46°35.880’	13°08.208’	1459	VK-153(v)
					VK-154(v)
	18	46°35.871’	13°08.210’	1448	VK-151(v)
	19	46°35.850’	13°08.195’	1474	VK-146(v)
					VK-161(v)
	20	46°35.793’	13°08.146’	1476	VK-135(v)
	21	46°35.808’	13°08.099’	1484	VK-131(v)
					VK-139(v)
V	22	46°35.729’	13°08.031’	1505	VK-136(v)
					VK-148(v)
	23	46°35.716’	13°08.001’	1534	VK-062(v)
					VK-134(v)
	24	46°35.713’	13°07.974’	1525	VK-160(v)
	25	46°35.677’	13°08.040’	1547	VK-155(v)
VI	26	46°35.759’	13°07.983’	1498	VK-133(v)