

Original Article

Elastic vipers: reproducing snakes adjust their size and internal volume to accommodate their developing offspring

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ABSTRACT

In many taxa, natural selection favours the ability of a female to accommodate a clutch or litter that is very large relative to her own body, placing a selective premium on traits that increase available abdominal space relative to litter volume. Flexible changes during pregnancy might help to mitigate these constraints. Using ultrasound scanning of captive snakes and snakes captured in the field, we explored such traits in reproducing female vipers (*Vipera aspis*). First, the anteriormost embryos moved forwards as they swelled during pregnancy, taking up space previously occupied by maternal viscera. Second, the oviductal membrane-bound packages containing embryos changed shape to fit flexibly into thicker vs. thinner parts of the mother's body. Third, intervertebral spaces increased during pregnancy, allowing the mother's body to lengthen. Maternal size elongation during pregnancy was reversed shortly after parturition. The decrease in size was closely related to the degree of abdominal displacement and reproductive output and was also evident in field-collected females of three European snakes. In summary, our data suggest that elasticity of the mother's body and flexibility in packaging of the offspring can mitigate abdominal constraints on maximal litter volume and space competition among siblings.

Keywords: body size; burden; elasticity; reproductive effort

INTRODUCTION

All else being equal, producing a larger number and/or mass of offspring per reproductive bout will increase the total lifetime output of progeny by a female and therefore be favoured by natural selection (Pincheira-Donoso and Hunt 2017). The evolutionary benefit of traits that enable a female to produce a large clutch relative to her own body size can be especially great if the costs of reproducing (e.g. cessation of feeding, vulnerability to predation) are independent of fecundity, rendering each offspring from a small clutch more 'expensive' to maternal fitness than an identical offspring from a larger clutch (Bull and Shine 1979, Ladyman *et al.* 2003). The advantages of accommodating a large clutch can also be increased if other factors constrain the opportunity to reproduce frequently; for example, if reproduction requires prolonged retention of progeny inside the mother's

body, in an environment where seasonality precludes multiple reproductive events per year. Both these conditions are satisfied in viviparous squamate reptiles living in seasonally cold areas (Lourdais *et al.* 2002a, 2004).

The ideas reviewed above suggest that the bodies of females will evolve to accommodate as large a clutch as is physically possible within the body. In keeping with that hypothesis, strong correlations between maternal abdominal volume and reproductive output have been documented in taxa such as insects (Wickman and Karlsson 1989) and reptiles (Forsman and Shine 1995, Shine 2000, Olsson *et al.* 2002). Importantly, embryo volumes increase over pregnancy, requiring rapid responses to the volumetric challenges of reproduction resulting notably from massive water intake during the period of fetal growth (Qualls and Shine 1995, Lourdais *et al.* 2015). Experiments that have

manipulated available space within the maternal abdomen (by inserting foreign objects to take up space) support a causal role for abdominal volume in constraining reproductive output in lizards (Du and Lü 2010).

How might natural selection (and/or fecundity selection) increase the capacity of a female physically to accommodate a large litter within her body? We can envisage several mechanisms (Table 1), as follows: (i) changes to embryonic packaging (reduction in water content of the embryonic sac fluids); (ii) flexibility in shape of the embryonic package to use the available space efficiently; or (iii) an increase in available abdominal space for the litter. Within this third category, we can distinguish two suites of traits. The first possibility contains characteristics that are constitutively expressed, differ between conspecific males and females, and are evident year-round (Shine 2000). The second possibility includes traits that are expressed only during pregnancy (ability to distend the abdomen, to elongate the trunk or to allow embryonic material to extend into parts of the abdomen usually filled by viscera). In total, then, adaptations of both reproductive investment and maternal physiology might reduce the degree to which physical constraints limit reproductive output (Table 1).

Previous research has provided evidence for some of the traits listed above. Notably, an extensive literature invokes fecundity selection (advantages of a larger clutch size) as the selective advantage for larger body sizes of females than conspecific males in many species (Shine 1988a) and for relatively larger length of the abdomen relative to overall body length in females than in males (e.g. Olsson et al. 2002). Likewise, females of most oviparous species of reptiles oviposit when the embryos are relatively small, and most water uptake will occur during incubation; hence the total mass of the embryo is far lower than it will be at hatching (Shine 1983, Shine et al. 2018). We also expect natural selection to optimize the volume and mass of the oviductal package relative to the size of the offspring that it will produce, and the most effective way to do so is to reduce water content (Meiri et al. 2020). Given that constraints of space are likely to be greatest for females that carry their offspring through to full development, we might expect water economy to be most evident in viviparous species. Comparative studies show that

viviparous squamates allocate less water to the embryo relative to energy content than do oviparous squamates (Bonnet et al. 2017), resulting in a similar total mass per oviductal offspring in species adopting either reproductive mode (Meiri et al. 2020).

To date, three of the potential routes for increasing abdominal volume during reproduction have not been assessed empirically. These flexible changes involve the following factors: (i) maternal trunk elongation; (ii) modification of the position of the embryonic packages; and (iii) modification of the shape of the embryonic packages. In combination, these traits should provide a robust way to mitigate internal volume limits. In the present study, we use high-resolution ultrasound to address these changes during pregnancy in a viviparous snake species that reproduces at low frequency but invests in large litter mass.

MATERIALS AND METHODS

Study species

The aspic viper, *Vipera aspis*, is a small viviparous snake of the western Palearctic region. The species is a typical capital breeder (Lourdais et al. 2002a), producing large yolky ova (Fig. 1). Pregnancy occurs during summer, lasts ≤ 3 months and is associated with sustained thermoregulation (Saint Girons 1952, Naulleau 1979, Ladyman et al. 2003), reduced movement (Lorionux et al. 2013b) and reduced food intake (facultative anorexia). During pregnancy, the female's abdomen becomes highly distended owing to an increase in the water content of the developing embryos (Dupoué et al. 2015a, Lourdais et al. 2015, Bonnet et al. 2017). Litter sizes range from 1 to 22 [mean ± SEM 5.85 ± 1.64; see Lourdais et al. 2003], and reproductive effort is high, with relative litter mass sometimes equalling postpartum maternal mass (Bonnet et al. 2003). Reproductive effort can vary among females within the same population or in the same female among years (Lourdais et al. 2002a, Dupoué and Lourdais 2014).

Capture and housing

In May 2012, we collected 27 pregnant female vipers in the Loire Atlantique district in west-central France. Reproductive state (late vitellogenesis) was determined initially by manual

Table 1. Possible mechanisms to increase the ability of a female reptile physically to accommodate a large volume of developing offspring, thereby allowing her to produce a large mass of neonates

Mechanism	Reference
(i) Reduction in size of offspring during development <i>in utero</i> relative to size at birth	
Deposit egg at early embryogenesis, before it swells in size	Shine (1983)
Reduce water content per offspring in large clutches, and with shift to viviparity	Bonnet et al. (2017), Meiri et al. (2020)
(ii) Increased flexibility of shape of offspring package	
Reduce thickness of inflexible eggshell and other membranes	Blackburn (1998); this study
(iii) (a) Increased abdominal volume of mother (sexually dimorphic traits)	
Increased overall body size of mother	Shine (1988a)
Increased abdomen length relative to overall body length	Olsson et al. (2002)
(iii) (b) Increased abdominal volume of mother during pregnancy	
Ability to distend skin, thereby increasing body diameter	Lourdais et al. (2017); Tomasheski et al. (2003)
Ability to stretch vertebral units, thereby increasing abdominal length	This study
Ability to allow developing embryos to displace viscera	This study

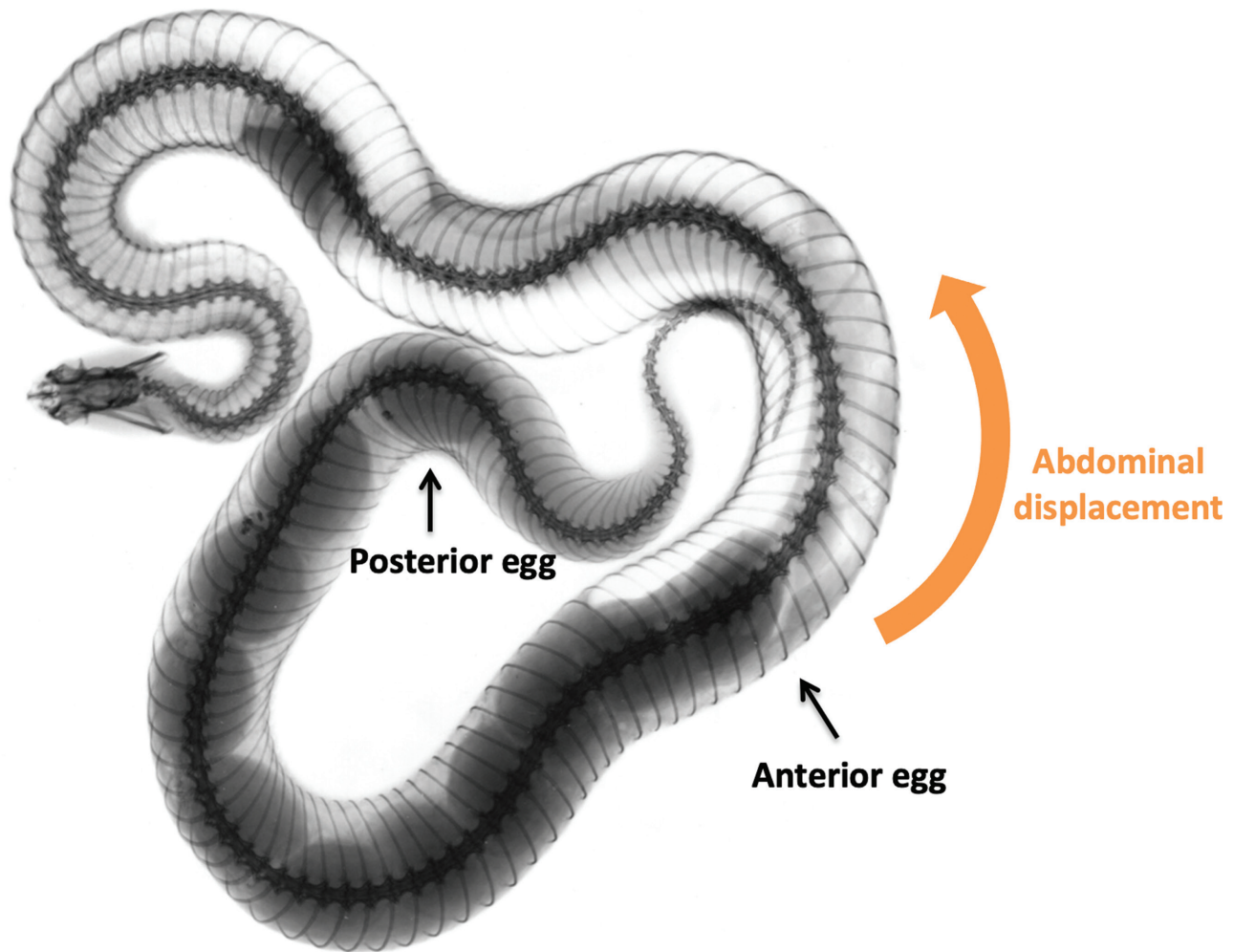


Figure 1. Dorsal view (X-ray) of a female *Vipera aspis* at the onset of pregnancy. A total of 11 recently ovulated eggs can be identified. The orange arrow illustrates movement of the anteriormost egg (abdominal displacement) in relationship to the increase in embryonic volume throughout pregnancy.

palpation of the abdomen and confirmed in the laboratory with high-resolution ultrasonography (SonoSite microMaxx, Bothell, WA, USA). Three females with undeveloped ova were removed from the study. We recorded initial body mass (mean \pm SEM 100.5 ± 0.68 g) and initial snout-vent length (SVL; mean \pm SEM 46.5 ± 0.69 cm). Female vipers were housed individually in cages (100 cm \times 30 cm \times 35 cm) that had a thermal gradient (18–41°C) created by a 75 W incandescent light bulb suspended over one side of the cage. The light was on for 6 h per day, enabling basking from 10.00 to 16.00 h, but forcing body temperatures to fall to room temperature (18°C) at night. Each cage contained a shelter (half-cylinder of PVC pipe, diameter 15 cm, length 35 cm, with two 3 cm openings in the sides) at the opposite end of the cage from the basking zone. Females were not fed until parturition, to mimic natural conditions (Lourdais *et al.* 2002b).

Reproduction dynamic and variables measured

We defined the following three phases of the reproductive cycle, based on overt changes plus high-resolution ultrasonography (Lourdais *et al.* 2015): (i) ovulation: pre-ovulation ecdysis (a reliable indicator of the onset of gestation in this species; Lourioux

et al. 2013a) was considered as the ovulation date and confirmed using ultrasound scans; (ii) late pregnancy: based on regular ultrasound scans, we defined 'late pregnancy' as the stage when embryos were fully developed (on average, 78.4 ± 1.1 days after ovulation); and (iii) post parturition: data on morphology of postpartum females were collected within 24 h after they gave birth.

Female body size

The SVL and tail length (TL) (± 1 mm) were determined by holding the snake's head and gently lining up the body along a tape measure. This metric was always collected by the same operator (O.L.), using limited traction force and waiting until the animal was immobile and had ceased resisting the pressure. For each individual in each measuring session, we took three measurements of SVL and TL; the results were highly repeatable (all $r > .90$).

Volume of the embryonic package

Viper embryos develop *in utero* inside separate membrane-bound packages equivalent to shell-less eggs. We monitored changes in volume of the embryonic package (i.e. embryo plus yolk plus

fluids and extra-embryonic membranes) to estimate water uptake during pregnancy. Using high-resolution ultrasonography, we determined the total volume at two stages of embryonic development (Hubert and Dufaure 1968): ‘ovulation’ (1.9 ± 0.7 days before ecdysis) and ‘late pregnancy’ (55.5 ± 1.3 days after ecdysis; $70.6 \pm 1.5\%$ of developmental duration). For each female, we collected frontal-view images of the anteriormost and posteriormost embryonic packages and measured their heights (H), lengths (L) and shape. We estimated package volume (in cubic centimeters) following the method described by Maritz and Douglas (1994), which combines information on L and H of an embryonic package and a coefficient associated with its form (F_{emb}): embryonic package volume = $\pi \times L \times H^2 \times F_{\text{emb}}$. For analysis, we averaged the volume of two embryonic packages measured at each stage for each female.

Position of the first embryo

We assessed the position of the anteriormost embryonic package at early and late pregnancy. We aligned the body of a pregnant snake to a tape measure and moved the ultrasound probe along the body to measure the distance (± 5 mm) between the maternal snout tip and the embryo. The difference in position (in centimetres) between late and early pregnancy provided a measure of abdominal displacement of the litter (hereafter ‘AD’; see Fig. 1).

Vertebral unit length

We used high-resolution ultrasound to look for intervertebral stretching. A probe was positioned parallel to the anteriormost and posteriormost embryonic packages to observe the spine and a group of ~ 15 vertebrae. When the frontal view of the spine was optimal, an image was collected. Then we determined the length (in centimetres) of a vector spanning eight vertebrae to encompass the length of the spine adjacent to the embryos (our reference point was the transverse process, which is highly echogenic; see Supporting Information, Fig. S1). We divided this measurement by seven to estimate the mean length per vertebral unit (i.e. one vertebra plus one intervertebral space). Measurements were carried out at ovulation, during late pregnancy and shortly after parturition (to ensure accurate positioning postpartum, we made marks with a sharpie permanent marker on the skin of the pregnant female to indicate the position of anterior and posterior embryos).

Reproductive output

For each female, we collected data on litter size (combining the number of neonates and fully developed stillborns); litter mass (mass of neonates and fully developed stillborns); for details, see Lourdaïs et al. (2002b). Stillborns were included in the analysis because they reached full development and therefore contributed to volumetric constraints during pregnancy. We also calculated the relative litter mass at parturition (ratio of litter mass to postpartum maternal mass) because this variable is a widely used descriptor of reproductive output in squamate reptiles (Bonnet et al. 2003, Dupoué and Lourdaïs 2014).

Evidence from free-ranging snakes

To see whether snakes exhibit intervertebral stretching in the wild as well as in captivity, we examined data on changes in the body size of individuals that were captured shortly before giving

birth and held in captivity only briefly (average 2 weeks) before being remeasured postpartum. We have such data not only for *V. aspis* ($N = 19$) but also for *Vipera berus* ($N = 21$) and *Coronella austriaca* ($N = 18$) from a long-term survey of sympatric populations in western France (Loire Atlantique) (Guiller et al. 2012, 2014). Snakes were captured in late summer shortly before parturition, then maintained in captivity in conditions similar to those described above. The same person (G.G.) measured SVL and TL (0.1 cm) at capture and shortly after parturition (24 h).

Statistics

All statistical analyses were carried in R (v.4.0.2) and R studio (v.1.3.959) using the packages ‘nlme’, ‘AICcmodavg’ and ‘emmeans’.

Embryonic position and volume

We first examined variation in position of the anteriormost embryo using a linear mixed model, with embryo position (in centimeters) as the response variable, time period (early or late pregnancy) as a binary fixed factor, maternal SVL at the corresponding session as a linear covariate and individual identity as a random factor. Second, we analysed embryo displacement in the abdomen (AD = difference between position taken in late vs. early pregnancy) using linear models. We tested the combined effects of initial maternal SVL and size-adjusted fecundity (residuals from the regression between litter size and maternal SVL). We then used the ‘step()’ function for a stepwise selection of the best model. Finally, we analysed variation observed in embryonic package volume (in centimetres cubed), length or width (in millimeters) in separate linear mixed models, including embryonic position (anterior vs. posterior), time period (early vs. late in pregnancy) and the interaction term as explanatory variables. Individual identity was set as a random factor to account for repeated contributions. We used the anova() function set to a ‘marginal’ type to obtain an equivalence of type III sums of squares.

Variation in vertebral unit length and maternal size

We use a linear mixed model to analyse the length of vertebral units (in millimetres), testing the influence of position (anterior/posterior), time period (early/late/postparturition) and the interaction term. Individual identity was included as a random factor. We used a similar procedure to analyse maternal SVL (in centimetres) and TL (in centimeters) over the same three time periods. We also analysed changes in maternal SVL (in centimeters) over pregnancy (difference between measures taken in late vs. early pregnancy) and at parturition (difference between measures taken postparturition vs. in late pregnancy). We used the anova() function set to a ‘marginal’ type to obtain an equivalence of type III sums of squares.

Determinant of reproductive output

We examined variation in reproductive output considering litter mass (mass of live neonates and fully developed stillborns) and relative mass change at parturition (ratio of litter mass to postpartum maternal mass) as response variables. We tested the influences of maternal SVL (postparturition), SVL changes at parturition and AD. Given that AD was correlated with maternal body size, we first extracted residuals from the regression

between embryo linear displacement and maternal SVL. For each response variable, we compared six models, including a constant model ($Y \sim 1$), three models with a single explanatory variable ($Y \sim \text{SVL}$, $Y \sim \text{AD}$ and $Y \sim \text{SVL changes}$) and two models with combined effects ($Y \sim \text{SVL} + \text{AD}$ and $Y \sim \text{SVL} + \text{SVL changes}$). We used the corrected Akaike information criterion (AICc) to select the most appropriate model (ΔAIC with the best model below a threshold of two).

Evidence from the field

Variation in maternal SVL and TL of recently field-caught female snakes at the time of parturition were analysed in separate linear mixed models testing the influence of species (*V. aspis*, *V. berus* and *C. austriaca*), time period (late pregnancy/postparturition) and the interaction term. Individual identity was treated as a random factor. Tukey's post hoc tests were conducted for 2×2 comparisons. Unless otherwise stated, values are reported as means \pm SEM.

RESULTS

Displacement of the anterior embryo

The distance from the mother's snout to the anterior embryonic package was positively related to maternal SVL ($F_{1,21} = 23.88$, $P < .001$). Anterior embryonic position decreased over time, from 26.87 ± 0.59 cm at early pregnancy to 21.34 ± 0.45 cm at late pregnancy ($F_{1,21} = 5.16$, $P = .03$), and this decrease was more marked for longer females (interaction term: $F_{1,21} = 12.45$, $P = .002$; Fig. 2A). The resulting AD ranged from 0.9 to 9.9 cm (mean = 5.53 ± 0.39 cm) which represented $\leq 19\%$ of female body length. We found that AD was best explained by combining the influences of initial maternal SVL ($\beta = 1.10 \pm 0.27$, $F_{1,21} = 17.10$, $P < .001$) and adjusted fecundity ($\beta = 0.46 \pm 0.14$, $F_{1,22} = 10.87$, $P = .003$; Fig. 2B).

Variation in shape of the embryonic package

The mean volume of the embryonic package did not differ between anterior and posterior locations ($F_{1,66} = 0.42$, $P = .51$), but increased through time (from early to late pregnancy, 4.94 ± 0.18 vs. 9.24 ± 0.31 cm³, $F_{1,66} = 80.89$, $P < .001$) irrespective of position (interaction term: $F_{1,66} = 0.59$, $P = .44$). Changes in the length and width of the embryonic package were affected by the location within the mother's body (interaction between reproductive stage and position: length $F_{1,66} = 7.34$, $P = .008$; width $F_{1,66} = 5.32$, $P = .02$). Caudal embryonic packages became longer ($+1.93 \pm 0.19$ cm, Tukey's test, $P < .001$) but not wider ($+0.03 \pm 0.04$ cm, Tukey's test, $P = .92$) during pregnancy, whereas anterior embryonic packages increased in both length ($+1.19 \pm 0.19$ cm, Tukey's test, $P < .001$) and width ($+0.17 \pm 0.04$ cm, Tukey's test, $P < .0023$).

Changes in vertebral unit length and maternal length

We detected a significant influence of reproductive stage ($F_{2,115} = 30.02$, $P = .001$) and position within the maternal body ($F_{1,115} = 144.58$, $P < .001$) on vertebral unit length, in addition to an interaction between the two factors ($F_{2,115} = 4.18$, $P = .017$). Average vertebral length unit increased over pregnancy ($+86.05 \pm 3.44$ μm , Tukey's test, $P = .001$; Fig. 3A) but decreased soon after parturition (-153.8 ± 3.19 μm , Tukey's

test, $P < .001$; Fig. 3A). Variation in maternal SVL mirrored the above-reported changes in elongation of the vertebral units ($F_{2,46} = 44.35$, $P < .001$). Maternal SVL increased from ovulation to late pregnancy ($+0.45 \pm 0.05$ cm), Tukey's test, $P < .001$; Fig. 3B) and decreased from late pregnancy to postparturition (-0.44 ± 0.05 cm, Tukey's test, $P < .001$; Fig. 3B). Initial and final SVL values did not differ (Tukey's test, $P = .98$, Fig. 3B). The changes in SVL during pregnancy were not significantly related to initial female SVL ($F_{1,22} = 1.39$, $P = .15$). Likewise, SVL changes at parturition were not related to postpartum SVL ($F_{1,22} = 0.04$, $P = .97$). Maternal TL did not differ across reproductive stages ($F_{2,46} = 1.32$, $P = .28$, 6.24 ± 0.11 , 6.18 ± 0.11 and 6.23 ± 0.12 cm for initial, late pregnancy and postparturition, respectively).

Determinants of reproductive output

The number of fully developed neonates produced per litter ranged from 1 to 11 (5.16 ± 0.47). Litter masses ranged from 9.65 to 54.02 g (29.64 ± 2.44 g) and represented 14–98% ($53.04 \pm 3.67\%$) of female body mass postpartum. Variation in litter mass was best explained by the combined influence of postparturition maternal length (SVL) and changes in SVL (Table 2). The best model to explain variation in relative litter mass retained changes in maternal SVL at parturition (Table 2, Fig. 4). However, the model combining SVL and changes in size was close to the threshold of two AIC units (Table 2). Change in maternal length was a better predictor of reproductive output than was the magnitude of AD of embryonic packages.

Evidence from free-ranging snakes

Mean maternal SVL differed among species ($F_{2,54} = 29.19$, $P < .0001$), with *C. austriaca* being shorter than *V. berus* (Table 3). Parturition was associated with a decrease in maternal body length (effect of reproductive stage: $F_{1,54} = 14.58$, $P = .0003$) regardless of species (interaction term: $F_{2,54} = 0.99$, $P = .37$). The reductions in SVL were -1.07 ± 0.18 , -0.82 ± 0.17 and -0.72 ± 0.16 cm for *V. berus*, *V. aspis* and *C. austriaca*, respectively (Table 3). Tail length differed among species ($F_{2,54} = 97.16$, $P < .0001$) but did not change over parturition (effect of reproductive stage: $F_{1,54} = 0.02$, $P = .87$) for all species (interaction term: $F_{2,54} = 0.18$, $P = .83$; Table 3).

DISCUSSION

In order to maximize lifetime reproductive success, especially if some of the costs of reproduction are incurred regardless of fecundity (Ladyman et al. 2003, Llorix et al. 2013b), a female squamate reptile is under selection to maximize her reproductive output per bout. As a result, physical constraints of abdominal volume become important. Potential solutions to that challenge might include making the package flexible such that it can readily deform to fit into available space or expanding the space available within the maternal abdomen by allowing embryos to extend over a greater proportion of the trunk and by lengthening the trunk (via elongation of the spine). Our study of pregnant asp viper provided data in support of all three hypotheses. We found dynamic yet reversible adjustments in abdominal volume and spinal elongation, in ways that increase the total volume for developing offspring within a female's body.

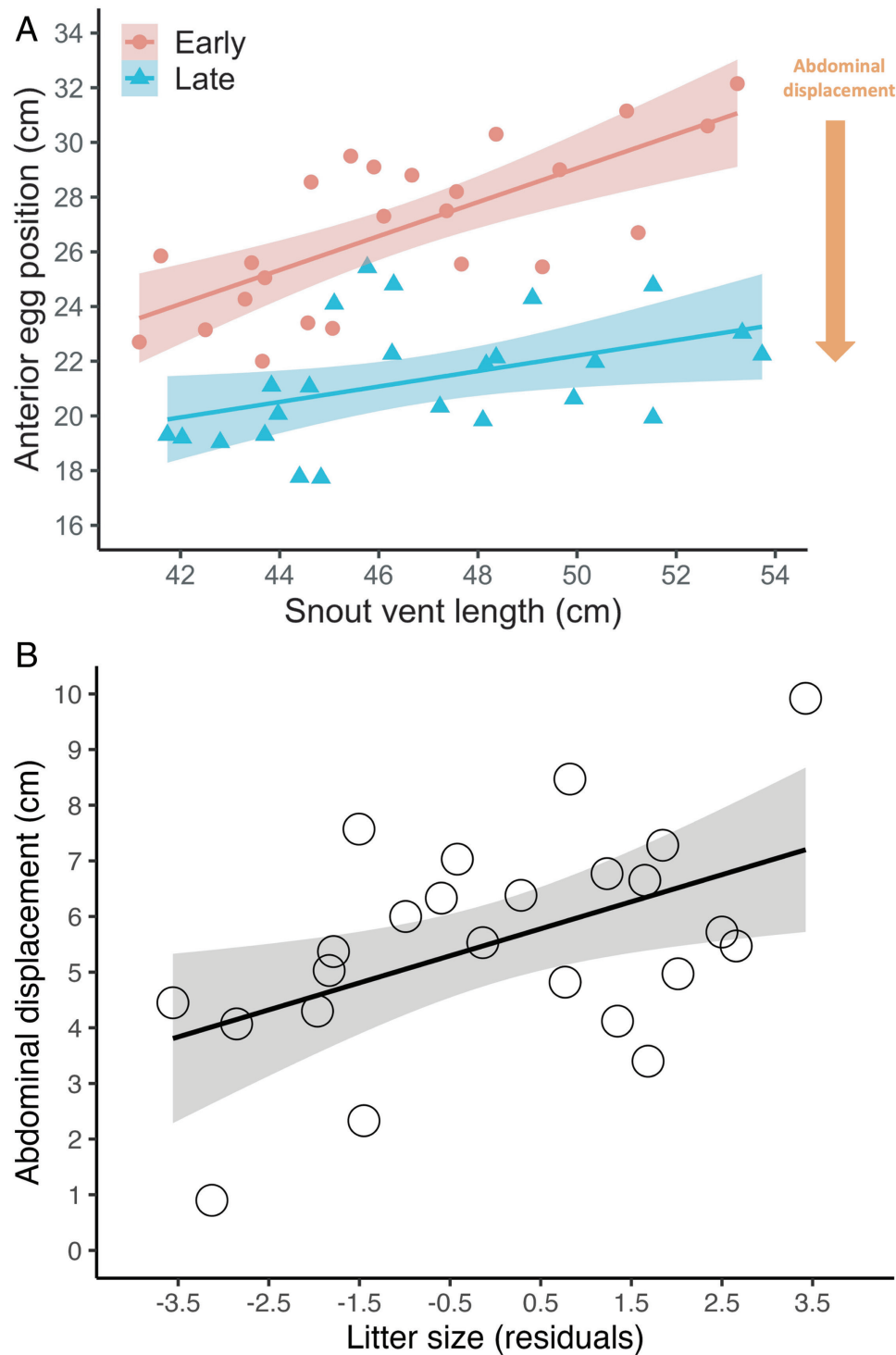


Figure 2. A, relationship between maternal snout-vent length (SVL; in centimeters) and the position of the anterior egg (in centimetres) in early pregnancy (pink circles) and late pregnancy (light blue triangles). The line represents the fitted linear regression and the shaded areas the 95% confidence interval. B, the influence of litter size (number of neonates) on absolute abdominal displacement (in centimeters), which represents the shift in position of the anteriormost egg within the mother's body. Litter size was adjusted for maternal SVL (residuals). The line represents the fitted linear regression and the grey area the 95% confidence interval.

We found that the first embryo moved anteriorwards ($\leq 19\%$ of maternal body length) into areas occupied by viscera before pregnancy. This shift was closely related to the number of developing embryos and their additive volume increase. Each embryonic package conformed to local space constraints within

the maternal abdomen (e.g. more slender in thinner parts of the maternal body). Also, female vipers stretched their spine in a way that increased the effective length of the trunk region, thereby increasing the space available for the developing offspring. No changes were observed in tail length, supporting the hypothesis

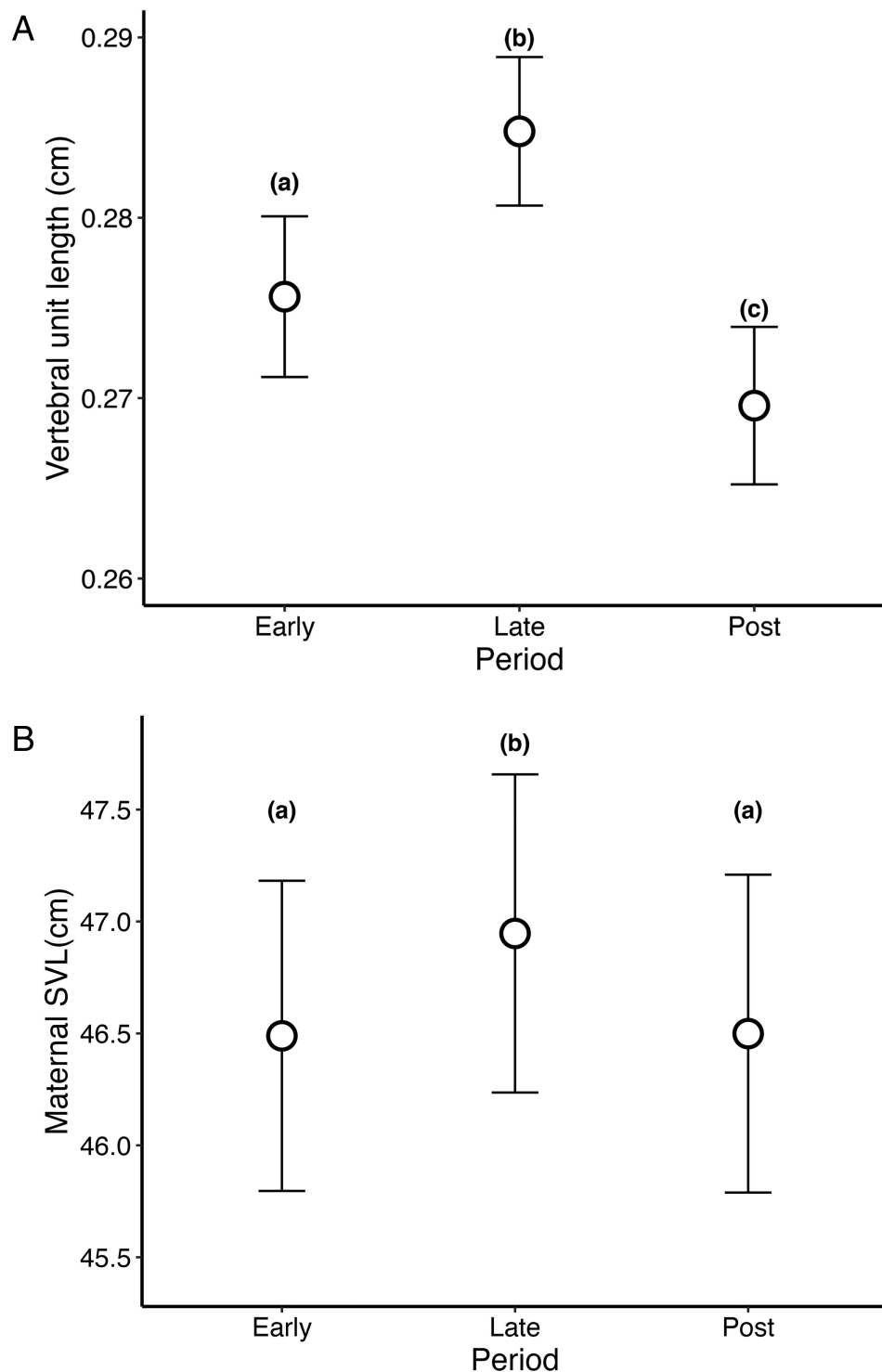


Figure 3. A, vertebral unit length (in centimeters) measured at ovulation (“Early”), at the end of pregnancy (99 ± 0.33 days; “Late”) and after parturition (within 24 h; “Post”). Vertebral unit measurements were estimated from a segment combining seven vertebrae and the associated seven intervertebral spaces. B, maternal snout–vent length (SVL; in centimeters) measured at ovulation (“Early”), at the end of pregnancy (“Late”) and after parturition (“Post”). Different letters indicate significant differences between groups.

that spinal flexibility was related to abdominal burden and not a measurement artefact (Luiselli 2005).

Similar flexibility might be common in other types of animals, but taxa presumably differ in the degree to which modifications of maternal morphology can accommodate a large volume of

reproductive material within the abdomen. Research on humans provides strong evidence of volumetric constraints on carrying the developing embryo: other internal organs are displaced by the fetus as pregnancy progresses, and mothers make postural adjustments (Whitcome *et al.* 2007, Biviá-Roig *et al.* 2019) that

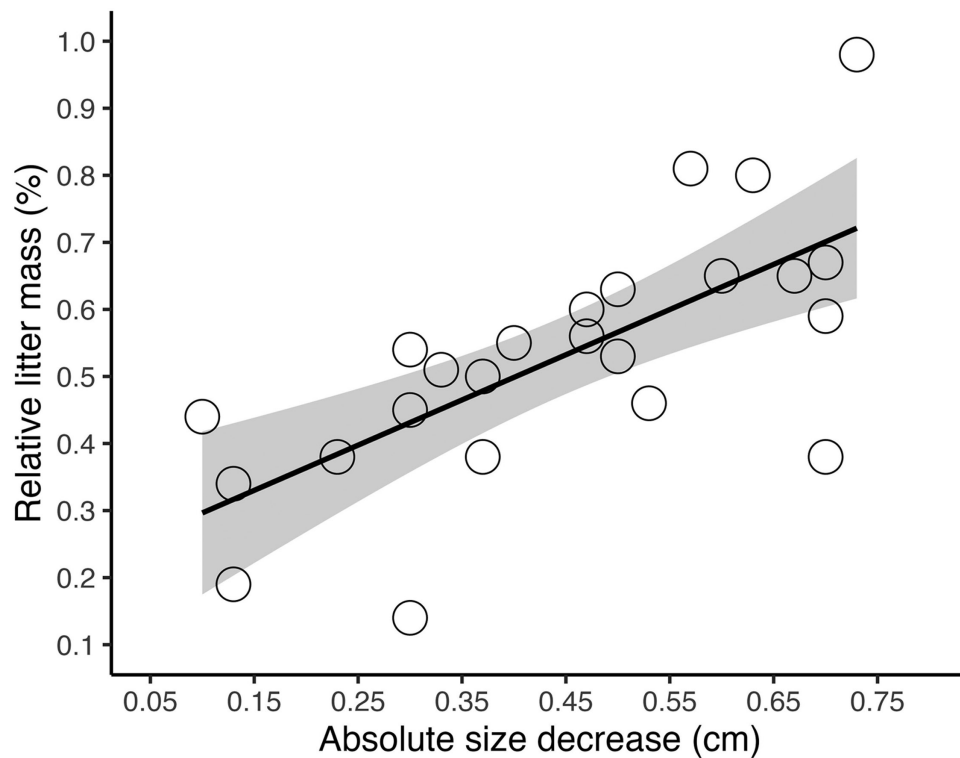


Figure 4. Relationship between absolute decrease in maternal snout–vent length (SVL) at parturition and relative litter mass (the ratio of litter mass to female postpartum body mass). The line represents the fitted linear regression and the grey area the 95% confidence interval.

Table 2. Determinants of reproductive effort, including litter mass (A) and relative litter mass (B). We tested the separate and combined influences of body size [snout–vent length (SVL)], size-adjusted (residuals) abdominal displacement (AD_{resid}) and size changes (ΔSVL) at parturition (SVL changes). We used stepwise model selection based on the corrected Akaike information criterion (AICc) to select the most appropriate model. K refers to the number of parameters and w_i to the AICc weight.

Model	k	AICc	$\Delta AICc$	w_i	Log-likelihood
(A) Litter mass					
SVL + ΔSVL	4	168.7	0	0.99	−79.3
SVL + AD_{resid}	4	177.7	9.01	0.01	−83.8
SVL	3	179.72	11.03	0	−86.26
ΔSVL	3	186.99	18.17	0	−89.83
Null	2	190.94	22.24	0	−93.18
AD_{resid}	3	191.04	22.35	0	−91.92
(B) Relative litter mass					
ΔSVL	3	−21.81	0	0.74	14.51
SVL + ΔSVL	4	−19.64	2.17	0.25	14.87
AD_{resid}	3	−12.98	8.84	0.01	10.09
SVL + AD_{resid}	4	−10.59	11.23	0	10.35
Null	2	−8.53	13.29	0	6.55
SVL	3	−6.25	15.56	0	6.73

The selected model appears in bold.

expand thoracic volume (Bellemare et al. 2003) and modify lumbopelvic morphology (Whitcome et al. 2007). Invertebrates with flexible abdomens exhibit significant distension during

reproduction (Wintle and Reinhardt 2008, Starck et al. 2018), and even the hard exoskeleton of brachiuran crabs has enough elasticity to accommodate the clutch (Hines 1992).

Our data on snakes captured from the field shortly before parturition suggest that spinal flexibility is not a consequence of prolonged captivity and is found in other species. Snakes are of particular interest because many species (including vipers) ingest meals that are large relative to the body size of the predator, causing massive distension of the body (e.g. Glaudas et al. 2019). As a result, snakes of all age classes and both sexes might be under intense selection to be able to increase space flexibly within the trunk, by traits such as increased numbers of scale rows (Shine 2002) and increased elasticity of the skin in relevant regions of the body (Tomasheski et al. 2003). Hence, snakes might be pre-adapted to some of the morphological changes required to increase abdominal volume for a developing litter. Nonetheless, some ecotypes of snakes might be under selection to minimize bodily distension; for example, a slender body shape might facilitate locomotion through the trees and through underground tunnels (Pizzatto et al. 2007) or within narrow crevices (Goodman et al. 2009). Snakes that use arboreal habitats exhibit low reproductive output and adaptive modifications of internal anatomy that reduce distension by eliminating anterior–posterior overlap between ovaries on left and right sides of the body. In extreme cases, burrowing snakes exhibit the complete loss of one oviduct (Blackburn 1998). Likewise, the propulsive role of the hindbody in aquatic locomotion might impose strong fitness penalties on distension of this region, resulting in an anterior shift of the developing litter in aquatic snakes (Shine 1988b).

The structural changes that we found are related to embryonic water intake and the increase in volume (Lourdais et al., 2015).

Table 3. Variation in maternal snout–vent length (SVL; in centimeters) and tail length (TL; in centimeters) before and after parturition in 21 *Vipera berus*, 19 *Vipera aspis* and 18 *Coronella austriaca*. All females were captured in the field in late pregnancy. We provide the results of Tukey's tests for time effects for each species from the general model on morphological traits (SVL or TL). Individual identity was treated as a random factor. See main text for statistics.

Species	Preparturition SVL	Postparturition SVL	Tukey's test	Preparturition TL	Postparturition TL	Tukey's test
<i>Vipera berus</i>	52.07 ± 0.76	51.00 ± 0.74	<.0001	6.5 ± 0.70	6.61 ± 0.49	.97
<i>Vipera aspis</i>	52.08 ± 1.07	51.25 ± 0.98	.0006	6.73 ± 1.15	6.79 ± 0.62	.99
<i>Coronella austriaca</i>	50.08 ± 1.22	49.36 ± 1.24	.0044	10.02 ± 1.24	10 ± 1.25	.99

The selected model appears in bold.

Offspring water demand is high during pregnancy, resulting in a potential conflict over this resource with the mother (Dupoué *et al.* 2015b, Dezetter *et al.* 2021) and also among siblings (Bonnet *et al.* 2017). Maternal morphological adjustments reported here provide a robust way to alleviate these potential conflicts. Although modifications to the vertebrae during pregnancy are widespread in mammals (including rats, sheep, monkeys and humans), many of these cases might not be similar functionally to the situation that we have documented in vipers. In pregnant mammals, the most common change is a temporary increase in bone density and mineralization, which might function to counteract removal of minerals from the maternal system by the developing fetus, and for milk production after birth (Hood 2012). Neither of these reproduction-related demands apply to the lecithotropic vipers (Lourdais *et al.* 2002a), although the time course is broadly similar, i.e. most gains in bone density are lost soon after parturition (Bowman and Miller 2001). Perhaps the closest parallel to the viper situation involves spinal elongation in naked mole-rats. Well-established queens of this eusocial rodent have elongated bodies owing to enhanced rates of growth of lumbar vertebrae during early pregnancies (Henry *et al.* 2007). These changes allow females to carry large litters *in utero*, while remaining sufficiently slender to move through narrow burrows within the colony (Henry *et al.* 2007). Although the rodent example involves changes in vertebral shape (rather than simply in spacing) and is permanent rather than transitory, the selective advantage might be similar to that driving spinal elongation in pregnant snakes.

In vipers, the increase in spine length must be driven by inter-vertebral spaces, not by ossification of vertebrae per se, because the increased body length reverses immediately after parturition. This case is therefore unlike reports on Galapagos iguanas, in which the length of major bones can decrease during periods of prolonged food shortage (Wikelski and Thom 2000). Instead, the proximate mechanism in vipers might involve reversible hormonally induced effects on musculoskeletal structure. For example, increased rates of secretion of relaxin during pregnancy mediate lumbar adjustments and confer increased joint laxity that facilitates birth in women (Amarasekera 2012). More generally, the space between adjacent vertebrae in humans can vary with a circadian rhythm, reflecting forces imposed by upright vs. horizontal postures by day vs. at night (Green and Scott 2017). Increased ligament laxity is a plausible explanation for the pattern observed in vipers. Even if maternal morphology returns to pre-pregnant conditions soon after parturition, the physiological consequences of such adjustments might continue for a longer period, as observed in humans (Cherni *et al.* 2019). Thus, for

example, (Olsson *et al.* 2000) reported that the decrease in running speeds associated with pregnancy in lizards is manifested for at least a week after the animals give birth. The maternal responses that increase available abdominal volume to hold the developing litter might involve multiple pathways and multiple parts of the body, requiring significant time to reverse.

Future research

Our study extends a previous finding of morphological changes (abdominal skin distension; Lourdais *et al.* 2017) and reduced water allocation per offspring (Bonnet *et al.* 2017) in vipers. These combined results provide support to all five potential mechanisms to allow an increased total volume of developing offspring (Table 1). Future work could examine how the importance of these space-creating mechanisms varies across species and sexes. For example, we might expect that spinal elongation and skin elasticity would be more frequent in females if these traits evolved to accommodate reproductive requirements. We would also expect elongation but not skin distensibility in a species where bodily distension imposes negative fitness consequences (e.g. in arboreal, fossorial and aquatic snakes). Finally, the degree of flexibility in body volume should be highest in regions of the body where such distension fulfils an important role. In snakes, such fitness-relevant regions would include the stomach in both sexes (but only in species that consume large prey) and the hindbody in females (but not in the extreme rear part of the body in aquatic snakes, nor the overall body in arboreal and fossorial taxa). It would also be of great interest to clarify the proximate mechanisms that induce spinal elongation and skin distensibility; for example, do hormones associated with pregnancy directly affect these flexibility-conferring traits?

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

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AUTHOR CONTRIBUTIONS

O.L., A.D. and G.G. conceived the ideas and designed methodology. O.L., A.D. and G.G. captured snakes, contributed to experimental

procedures and collected the data. O.L. led the data analyses together with A.D. O.L. led the writing of the manuscript, and all authors contributed critically in interpretation of results and manuscript writing. All authors gave final approval for publication.

CONFLICT OF INTEREST

The authors declare no competing or financial interests.

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ETHICS

This study was performed in accordance with laws relative to capture, transport and experiments on *Vipera aspis*, *Vipera berus* and *Coronella austriaca* (DREAL permit no. 09/346 DEROG), and all procedures were approved by the relevant ethics committee (agreement no. A79-001 and capacity certificate no. 79-158).

DATA AVAILABILITY

Data are available in a public repository at <https://doi.org/10.5281/zenodo.8196079>

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