

Climate affects embryonic development in a viviparous snake, *Vipera aspis*

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Climatic conditions during embryonic development can exert profound and long-term effects on many types of organisms, but most previous research on this topic has focussed on endothermic vertebrates (birds and mammals). Although viviparity in ectothermic taxa allows the reproducing female to buffer ambient thermal variation for her developing offspring, even an actively thermoregulating female may be unable to provide optimal incubation regimes in severe weather conditions. We examined the extent to which fluctuations in natural thermal conditions during pregnancy affect reproduction in a temperate viviparous snake, the aspic viper (*Vipera aspis*). Data gathered from a long term field study demonstrated that ambient thermal conditions influenced (1) female body temperatures and (2) gestation length, embryo viability, and offspring phenotypes. Interestingly, thermal conditions during each of the three months of gestation affected different aspects of reproduction. Hotter weather early in gestation (June) increased ventral scale counts (= number of body segments) of neonates; hotter weather mid-gestation (July) hastened development and thus the date of parturition; and hotter weather late in gestation (August) reduced the incidence of stillborn neonates. The population that we studied is close to the northern limit of the species' range, and embryonic thermal requirements may prevent *Vipera aspis* from extending into cooler conditions further north.

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The environment affects living organisms in many ways. Influences that occur early in an organism's life – especially, while it is an embryo – typically have greater effects on its subsequent development than do influences that occur later in life (Henry and Ulijaszek 1996, Desai and Hales 1997). Recent research has documented many strong and persistent effects of environmental factors acting during embryogenesis on fitness-related traits (Lindström 1999, Lummaa and Clutton-Brock 2002). Most studies on this topic have been based on birds and mammals, reflecting the concentration of long-term individual-based studies on these taxa (Lindström 1999). However, we also need data on other kinds of

animals if we are to discern valid generalities about effects of the early environment on subsequent phenotypic traits. Ectothermic vertebrates are of particular interest in this respect. Because they contain both oviparous and viviparous taxa, often without the confounding influence of post-hatching parental care (Clutton-Brock 1991), they allow us to examine the degree to which alternative modes of reproduction buffer the developing offspring from environmental fluctuations.

Ambient temperatures not only fluctuate considerably through time, but they also influence ectothermic vertebrates in many ways. The most obvious influences concern variables such as the metabolic rates, activity

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levels and locomotor performance of adult animals (Huey 1982, Hertz et al. 1993). However, temperature also influences the rates and trajectories of ontogenetic development, and embryonic sensitivity to ambient temperature may be an important (albeit, less-studied) component of ectothermic biology. For example, extensive studies on oviparous reptiles show that the rate of embryonic development depends upon thermal regimes inside the nest. High temperatures greatly accelerate embryogenesis and thus, shorten the incubation period (Hubert 1985). Thermal regimes during embryogenesis can also profoundly affect phenotypic traits of the offspring, such as body size, scalation, and locomotor performance (Shine and Harlow 1996, Shine et al. 1997, Downes and Shine 1999, Andrews and Mathies 2000, Flatt et al. 2001, Shine and Elphick 2001, Webb et al. 2001). In some taxa, incubation temperatures directly determine the sex of offspring (Bull 1980).

The sensitivity of reptilian embryogenesis to ambient temperature suggests that thermal conditions may play a major role in the population ecology of these animals. For example, thermal minima for embryonic development may constrain oviparous species from reproducing in cold climates (Mell 1929, Weekes 1933, Tinkle and Gibbons 1977). More generally, geographic distributions of oviparous species may be set by the thermal requirements for embryogenesis (Shine 1987). Plausibly, annual variation in climatic conditions may influence the attributes of offspring (size, shape, time of hatching or birth) that enter the population each year, and thus influence the relative size of different year-classes. However, we are not aware of any data to show such an effect, apart from anecdotal reports of delayed oviposition, hatching or parturition in unusually cold years (Saint Girons 1952, Pengilley 1972, Olsson and Shine 1997). In contrast, a wide range of studies on endothermic vertebrates (especially mammals) have not only documented effects of climatic variation on neonatal phenotypes, but also have shown that the resulting effects have long-term consequences for survival and reproductive success of individuals from those cohorts (Albon et al. 1983, Post et al. 1997, Lummaa and Clutton-Brock 2002).

The influence of ambient thermal fluctuations on hatching dates and offspring phenotypes is likely to be obvious in oviparous species of reptiles, because females in such taxa cannot control the thermal conditions experienced by their offspring throughout post-oviposition development (except in cases of maternal egg-brooding, Vinegar 1973). We might expect that this sensitivity to ambient thermal regimes would be much lower in a viviparous (live-bearing) species. Viviparity has evolved > 100 times within squamate reptiles and this transition seems to have occurred primarily in cold climates (Blackburn 1985, 1999, Shine 1985). The probable selective force for these repeated transitions

has been the egg-retaining female's ability to maintain high, relatively constant incubation temperatures for her developing offspring (Shine 1985). Because the gravid female can regulate her temperature behaviorally, moving among microhabitats to exploit thermal heterogeneity in the environment, the temperatures experienced by an offspring developing in utero will be buffered considerably from fluctuations in ambient temperature (Shine 1983, Burger and Zappalorti 1988, Charland and Gregory 1990, Schwarzkopf and Shine 1991, Peterson et al. 1993).

Nonetheless, if ambient thermal conditions fluctuate considerably, even a carefully-thermoregulating viviparous female may be unable to maintain high, constant temperatures for her developing offspring. In keeping with this inference, laboratory studies that have manipulated basking opportunities for viviparous lizards have found many of the same phenomena as described above for egg-layers. That is, a viviparous female reptile's access to basking opportunities not only determines rates of embryogenesis (and thus, the duration of her pregnancy, Naulleau 1986, Schwarzkopf and Shine 1991) but also affects many phenotypic traits of her offspring (Shine and Harlow 1993, Swain and Jones 2000, Wapstra 2000, Arnold and Peterson 2002). Unfortunately, the relevance of these results to free-ranging animals remains unknown.

Although many authors have stressed the ability of gravid females in viviparous reptile species to provide relatively high stable temperatures for their developing offspring (see above references), less attention has been paid to situations where they may be unable to do so. That is, do severely cold or rapidly fluctuating weather conditions make it impossible for even an actively thermoregulating viviparous female to provide an effective thermal buffer for her offspring? In such a situation, we might expect to see the developmental rates and phenotypic traits of offspring respond to annual variation in weather conditions, despite the thermoregulatory efforts of their mothers. Such effects should be especially important for individuals living at the altitudinal or latitudinal margin of the geographic range of a species. We examined this possibility with data from a nine-year study of a free-ranging population of viviparous snakes at the extreme northern limit of the species' range.

Material and methods

Study animals

The aspic viper, *Vipera aspis* Linné, is a small viviparous snake of the western-Palaearctic region and is locally abundant at the northern limit of its distribution in France. Females mature at 40 cm snout-vent length (SVL), which is attained in 2.5 to 3.5 years (Bonnet et al. 1999a). Ovulation typically occurs during the first two

weeks of June with limited geographical variation (Saint Girons 1957, Naulleau 1981). During gestation pregnant females display higher thermal preferences and substantially increase basking times (Saint Girons 1952, Naulleau 1979, Bonnet and Naulleau 1996, Lourdaï et al. 2002b, Ladyman et al. 2003). Parturition occurs two to three months after ovulation, from late August through late September.

Study site and methods

The study site is near the village of Les Moutiers en Retz in west-central France (47°03N'; 02°00W'). It is a 33-hectare grove with a mosaic of meadows and regenerating scrubland. Details on the field site, methods and searching effort are available in related works (Bonnet et al. 2000, 2001, 2002). Previous results suggest that climatic conditions in this area not only prolong gestation by one to two months compared to warmer-climate (Mediterranean) populations, but also that the magnitude of such effects varies among years (Lourdaï et al. 2002a).

Gravid females were captured and maintained in captivity after the first parturition of the year was recorded in the field (generally in late August). Reproductive data were then obtained on 173 litters from 148 different females. For most individuals (127) only a single litter was obtained, but 17 females produced two litters and 4 individuals produced three litters.

The components of the litter were characterized (undeveloped ova, dead embryo, fully-developed but stillborn, healthy offspring), counted, and weighed (± 0.1 g). Young were measured (± 0.5 cm) and sexed. Stillborn offspring were measured, weighed, and sexed when possible. Because we could not distinguish unfertilized ova from ova that had been fertilized but had died early in embryogenesis, these were grouped in the same category (undeveloped ova). Using this method we gathered data on 817 healthy offspring, 132 undeveloped ova, 22 dead embryos and 78 stillborn offspring. From 1993 to 2000, ventral scales were counted for 136 mothers and 681 healthy neonates. Gestation period was calculated from parturition dates, assuming a fixed ovulation date of 10 June (Saint Girons 1980, Naulleau 1981).

Thermal conditions

In our study area, climatic conditions constrain many aspects of aspic viper ecology (Lourdaï et al. 2002a). These snakes are diurnal with prolonged basking episodes. Previous studies have revealed substantial daily variation in body temperatures, with low overnight temperatures followed by basking to attain and maintain high body temperatures during daylight hours. Pregnant

females bask much more than do non-pregnant animals, and thus are more often encountered and captured (Bonnet and Naulleau 1996, Lourdaï et al. 2002b). Thermal conditions fluctuate strongly from one day to the next in this temperate-oceanic climate. Does such variation affect the body temperatures of vipers, despite the buffering effects of behavioral thermoregulation? To answer this question we need measures of both ambient temperatures and viper body temperatures.

Ambient temperatures

As an index of ambient temperature we used daily maximum shaded air temperature, as measured in a standard meteorological shelter 1.8 m above the ground in Pornic (47°06N'; 02°07W'), near our field site (47°03N'; 02°00W'). These daily maxima will not necessarily have any close relationship to the actual temperatures experienced by an embryonic viper inside its mother's uterus, but instead should provide a rough index of a viper's opportunity for behavioral thermoregulation. Thermal maxima were generally achieved in the afternoon, one to two hours after sun zenith.

Aspic vipers have a long period of embryonic development (up to three months, Hubert and Dufaure 1968) and we distinguished three periods broadly corresponding to major steps in embryogenesis (Hubert 1985, Hubert et al. 1966, Hubert and Dufaure 1968): (1) early gestation (10 to 30 June), the onset of embryogenesis (including blastulation, gastrulation, neurulation, somite development, differentiation of the head and circulatory system); (2) mid-gestation (1–31 July), a period of organogenesis (development of the optic vesicles and olfactory bulb, appearance of the jaws, cloacal split, genitalia and trunk/tail scales; rapid growth of the embryo and development of spiral-coiling); and (3) late-gestation (1–31 August), a period of embryonic growth and completion of development (including development of pigmentation, and differentiation of head scales).

In our analysis, we calculated mean daily temperature maxima during each of these three periods of gestation for each year. We investigated the relative influence of each period by regressing the duration of gestation (as estimated from dates of birth: see above) against these mean temperatures. Then, we examined the effect of thermal conditions during development on offspring phenotype (scalation, snout-vent length). We did not deal with offspring body mass and body condition (mass relative to SVL) because previous work showed that these variables are strongly affected by annual fluctuations in food availability (Bonnet et al. 2001). Finally, we examined the possibility that embryo mortality rates might be influenced by thermal conditions during gestation.

Body temperatures of free-ranging vipers

Using internal temperature radiotransmitters (Naulleau et al. 1996), we monitored reproductive and non-reproductive female vipers during the gestation period (1 June to 31 August 1996). Females were sampled one to four times per day from 8:00 am to 9:30 pm, but to minimise temporal heterogeneity and pseudoreplication our analyses were based only on a single late-afternoon (5:00–8:00 pm) data point per female per day. Because of the 24-hour delay between successive readings (during which time the snakes' body temperatures dropped to minimum levels overnight, Naulleau 1997, pers obs), we have treated successive daily temperatures from the same individual as quasi-independent. Using this procedure 241 temperature records from 16 female Aspice vipers (9 reproductive and 7 non-reproductive) were available (average number of records per individual = 16 ± 7). The influence of daily maxima on female body temperature was examined after accounting for female identity and reproductive status.

Statistics

All statistics were performed with Statistica 6.0. Influences of ambient thermal conditions on female body temperature were examined using general linear regression modeling (GLM). Reproductive status was considered as a fixed factor. Female identity was treated as a random factor, nested within reproductive status. Daily temperature maxima or sampling dates (Julian calendar) were then treated as covariates (fixed effects). Influences of thermal conditions on offspring phenotype were investigated using mixed-model ANCOVAs. To account for correlated responses among offspring of individual litters and repeated female contributions (17 females reproduced twice and 4 three times), maternal identity was included as a random factor. Neonatal traits were the dependent variables, offspring sex was a fixed factor and annual thermal conditions were treated as covariates (fixed effects).

Results

Determinants of female body temperatures

GLM analysis suggested that female body temperature was affected by at least three factors (Table 1). First, our use of air temperature as an index of thermoregulatory opportunities was validated by a significant influence of maximum air temperature on female body temperature (Table 1). Second, a significant relationship between sampling date and daily thermal maxima reflected an increase in ambient temperatures over the summer period. Third, reproductive status also exerted a strong

Table 1. Determinants of female body temperature in free-ranging aspice vipers, *Vipera aspis*. Reproductive status (Status) was considered as a fixed factor. Females identity (Identity) was considered as a random factor and was nested in the corresponding reproductive status. Daily temperature maxima (Max) and Julian sampling date (Date) were then treated as covariates (fixed effect).

	Effect	df	MS	F	p-value
Status	Fixed	1	831.82	29.36	0.0001
Date	Fixed	1	307.55	10.85	0.001
Max	Fixed	1	403.24	14.23	0.0002
Identity	Random	14	169.54	5.98	0.0001
	Error	234			

influence on female body temperature with pregnant females maintaining higher body temperatures than did non-reproductive females ($28.7 \pm 0.5^\circ\text{C}$, $n = 133$ vs $25.47 \pm 0.6^\circ\text{C}$, $n = 117$, Table 1).

Fluctuations in thermal conditions

A repeated measure ANOVA (using year as the factor and gestation month within each year as the repeated factor) first indicated that mean daily air temperature significantly increased over the course of gestation ($F(2, 522) = 45.33$, $p < 0.00001$) and more importantly, that mean air temperature during gestation varied significantly among years ($F(8, 261) = 3.33$, $p < 0.0012$). We also detected a significant interaction between gestation month and year ($F(16, 738) = 4.53$, $p < 0.0001$), reflecting marked year to year fluctuations in thermal conditions over the three months of gestation (Fig. 1). During the study period, thermal conditions during gestation fell broadly into three patterns: parabolic (where the highest temperature was in July), sigmoid (where the predominant change was a major increase in temperature between June and July), and exponential (where the predominant change was a major temperature increase between July and August).

As a consequence, we found no significant correlation between monthly mean daily temperatures across years ($F(1, 7) = 1.52$, $n = 9$, $p = 0.25$ for June vs July; $F(1, 7) = 3.11$, $n = 9$, $p = 0.12$ for June vs August; $F(1, 7) = 0.01$, $n = 9$, $p = 0.93$ for July vs August). Therefore, we consider mean temperatures during each of the three months as independent variables for our subsequent analyses.

Impact on reproduction

Duration of gestation period

In a related study (Lourdais et al. 2002a), we showed that the duration of gestation in this population was influenced by mean temperatures during the gestation period, as well as by the frequencies of inviable elements in litters (i.e. undeveloped ova and stillborn offspring).

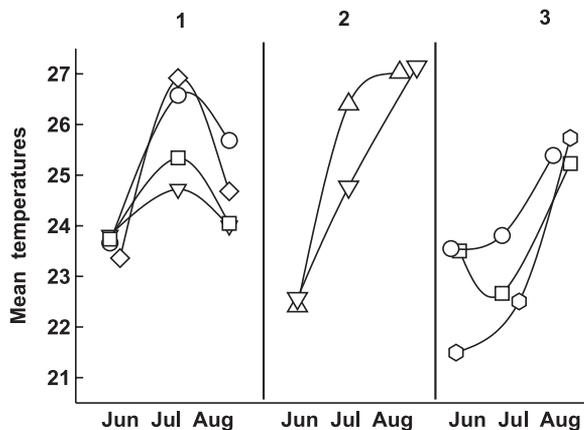


Fig. 1. Annual variation in thermal conditions at our study site in western France during the three months of gestation for asp viper (*Vipera aspis*). For simplicity, years were classified depending upon the thermal pattern observed. (Jun = June; Jul = July; Aug = August)

Pattern 1 (parabolic): 1992 (open triangles down), 1994 (open circles), 1996 (open squares), 1999 (open diamonds).

Pattern 2 (sigmoid): 1995 (open triangles up), 1997 (open triangles down).

Pattern 3 (exponential): 1993 (open squares), 1998 (open hexagons), 2000 (open circles).

For the present study, we can examine this result more closely in terms of the three phases of gestation defined above. We used stepwise multiple regression analysis for this purpose, and restricted the analysis to the 80 females that produced only viable offspring (i.e. no stillborns). Only mean daily temperatures during July (mid-gestation) were retained in the model (Table 2), accounting for 51.4% of the variance in overall gestation length.

Offspring phenotype

Scalation

First, we detected a strong influence of maternal identity on the number of ventral scales in newborn vipers (ANOVA, $F(101, 527) = 4.13$, $p = 0.00001$). This influence was partially attributable to significant heritability of ventral scalation, as we detected a significant relationship between maternal and neonatal number of ventral

Table 2. Influence of mean temperatures during the three months of pregnancy on the duration of gestation in female asp viper.

	Beta	Partial correlation	p-value
June	0.23	0.18	0.11
July	-0.77	0.62	< 0.0001
August	-0.09	0.09	0.44

scales ($r^2 = 0.11$; $F(1, 629) = 80.81$, $p < 0.0001$ treating each offspring as an individual point and $r^2 = 0.22$, $F(1, 114) = 34.64$, $p < 0.0001$ when considering mean number of ventral scales per litter). The two sexes differed in mean numbers of ventral scales, with neonatal females having more scales than their brothers (149.1 ± 0.17 , $n = 329$ vs 148.0 ± 0.17 , $n = 352$, $F(1, 563) = 11.37$, $p < 0.0007$, mixed model ANOVA using female identity as random factor and offspring sex as a fixed factor). Interestingly, we detected significant year to year variation in the number of ventral scales in neonatal snakes (ANOVA, $F(7, 673) = 12.400$, $p = 0.00001$, Fig. 2). This effect holds true even after accounting for maternal influence and offspring sex ($F(7, 114) = 3.08$, $p = 0.0004$, mixed model ANOVA using female identity as a random factor, offspring sex and year as a fixed factors). Such annual variations appear to be linked to the climatic fluctuations described above. For example, mean thermal maxima during gestation significantly affected the number of ventral scales in neonates ($F(1, 114) = 6.94$, $p = 0.008$, mixed model ANCOVA using female identity as random factor, offspring sex as a fixed factor and mean gestation thermal maxima as a fixed covariate). Because organizational effects of temperature are likely to occur early in embryogenesis, we re-conducted the analysis by considering each of the three components of the gestation period independently. Only mean daily temperature maxima during the first period (i.e. the three weeks following ovulation) led to significant results (Table 3, Fig. 3). This influence is reflected in a significant relationship between mean thermal maxima in June and the mean number of ventral scales in neonatal vipers ($r^2 = 0.49$; $F(1,6) = 7.75$ $p < 0.03$).

Snout-vent length

In contrast with offspring scalation, no significant year to year variation in offspring snout-vent length was detected ($F(8, 134) = 0.51$, $p = 0.84$, mixed model

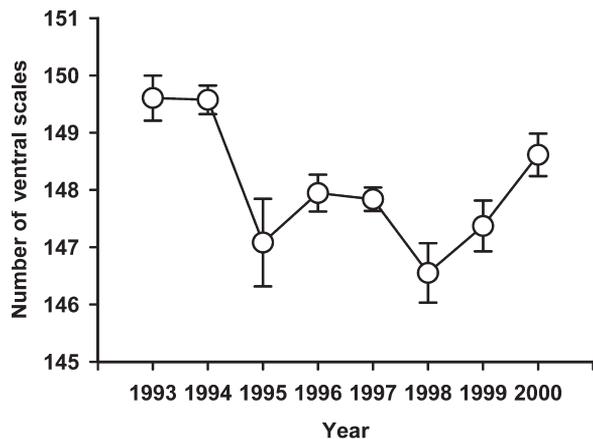


Fig. 2. Annual variation in number of ventral scales in neonatal

Table 3. Influences of climatic conditions during gestation (mean daily maxima calculated for each month), offspring sex (Sex), and maternal identity (Identity) on the number of ventral scales in neonatal aspik vipers.

	Effect	df	MS	F	p-value
June	Fixed	1	426.76	17.91	0.00001
July	Fixed	1	54.17	2.36	0.127
August	Fixed	1	14.43	0.60	0.44
Sex	Fixed	1	172.70	16.50	0.00001
Identity	random	116	24.5	3.40	0.00001
Error		560			

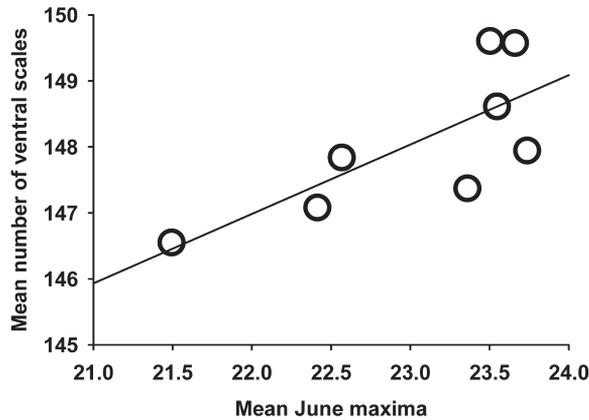


Fig. 3. Relationship between mean June ambient thermal maxima and mean number of ventral scales in neonatal vipers over the course of the study.

ANOVA using female identity as a random factor and year as a fixed factors). The two sexes did not differ significantly in snout-vent length ($F(1, 134) = 2.47, p = 0.11$, same design as above, but adding offspring sex as fixed factor). An offspring's SVL was significantly correlated with its number of ventral scales but explained very little of the variance in scale counts ($r = 0.19; r^2 = 0.04, n = 681, F(1, 681) = 26.58; p < 0.0001$). No significant relationship was found between thermal conditions and offspring SVL (Table 4).

Late embryonic death

In a previous analysis of reproductive output in this population (Lourdais et al. 2002a), we were not able to detect any significant relationship between mean temperature during the total gestation period (mid-June through August) and the production of inviable offspring (undeveloped ova, dead embryos plus stillborn offspring). Here, we focus on the production of stillborn offspring.

Stillborn offspring were produced in significant numbers ($n = 78$) during the study, with the proportion of females that produced dead offspring fluctuating significantly among years ($\chi^2 = 17.56; df = 8; p = 0.024$). Stillborn offspring were fully developed in appearance,

Table 4. Influences of climatic conditions during gestation (mean daily maxima calculated for each month) and maternal identity (Identity) on the snout-vent length of neonatal aspik vipers.

	Effect	df	MS	F	p-value
June	Fixed	1	5.31	1.03	0.31
July	Fixed	1	4.88	0.96	0.32
August	Fixed	1	4.93	0.04	0.82
Identity	random	135	0.59	8.59	0.00001
Error		675			

indicating that death occurred at late embryonic stages. They were significantly shorter (ANOVA, $F(1, 832) = 20.34, p < 0.00001$) and lighter (ANOVA, $F(1, 836) = 21.04, p < 0.00001$) than healthy neonates, suggesting that mortality was not a result of short-term maintenance of their mothers under laboratory conditions. Stillborn and healthy neonates did not differ in mass relative to SVL (ANOVA on residual scores, $F(1, 831) = 0.41, p < 0.52$). Mortality was not sex-biased ($\chi^2 = 0.24, df = 1, p = 0.62$, pooling the nine years of the study), nor did sex ratios of stillborn offspring vary significantly among years ($\chi^2 = 10.27, df = 8, p = 0.24$).

Based on these results, we looked for a possible influence of mean temperature during the latter part of gestation (July and August) on the probability of producing stillborn offspring. Excluding females producing undeveloped ova, we detected a significant negative influence of mean August temperature on the probability of observing late embryonic death (Logistic regression, $\chi^2 = 8.18, n = 113, p = 0.0042$). We also detected a significant negative relationship between mean August temperatures and the proportion of stillborn offspring ($r = 0.33, r^2 = 0.11, n = 113, F(1, 111) = 13.37, p < 0.0004$). The same analysis using mean July or mean June daily temperatures yielded non-significant results.

Discussion

Our relatively long-term field study demonstrates that natural climatic conditions influence important aspects of embryogenesis in the aspik viper. Although gravid vipers show distinctive thermoregulatory behaviors that result in relatively high, stable body temperatures throughout pregnancy (Saint Girons 1952, Naulleau 1979, Bonnet and Naulleau 1996, Ladyman et al. 2003), they are unable to completely buffer their developing embryos from year-to-year thermal variations in this relatively northern, cool-climate area. This result runs counter to the primary emphasis of published studies on thermoregulation by gravid reptiles, which have stressed the thermoregulatory precision of such animals (Shine 1983, Charland and Gregory 1990, Schwarzkopf and Shine 1991, Peterson et al. 1993). Clearly, this stenothermy is relative: even if gravid

females maintain higher temperatures than do non-reproductive conspecifics, they may still vary enough in body temperatures to impact on the embryos.

Unsurprisingly, high summer temperatures resulted in faster embryonic development and thus, earlier parturition dates. Thermal dependence of embryonic development is widespread in squamate reptiles (Blanchard and Blanchard 1941, Hubert 1985) and our results in this respect are consistent with an earlier experimental study conducted by Naulleau (1986) on this species and the closely related adder (*Vipera berus*). However, our data extend previous knowledge of this phenomenon in suggesting that the thermal sensitivity of gestation length seems to be significant mostly (or only) in the middle part of gestation (July), a period coinciding with major events in embryogenesis (i.e., organogenesis and active tissue synthesis).

Environmental conditions also had direct effects on the phenotypic traits of offspring. In keeping with laboratory studies that have manipulated basking opportunities for viviparous female reptiles and documented shifts in offspring phenotype as a result (Shine and Harlow 1993, Shine and Downes 1999, Swain and Jones 2000, Wapstra 2000, Arnold and Peterson 2002), we found significant correlations between ambient temperatures and phenotypic traits of the neonatal vipers. In contrast with the experimental study of Arnold and Peterson (2002) that documented a flat reaction norm for scale counts in another viviparous snake species (*Thamnophis elegans*), we detected a significant impact of temperature on ventral scalation in new born asp viper. Notably, mean temperature during early stages of embryogenesis affected the number of ventral scales in offspring, with higher temperatures increasing scale numbers. Similar influences of developmental temperature on scalation have been reported from laboratory experiments (Vinegar 1973, 1974, Osypka and Arnold 2000), and inferred from climate-correlated geographic shifts in scale counts (Klauber 1941).

In most snakes, the number of ventral scales is tightly correlated with the number of vertebrae, reflecting the number of pairs of somites differentiated during early embryogenesis (Hubert 1985, Lindell 1996). The number of ventral scales or body vertebrae shows considerable intraspecific variation (Lindell et al. 1993, Lindell 1996). If temperature influences somitisation, we would expect that differences in scalation (reflecting vertebral number) will be correlated with differences in body length (snout-vent length: SVL). During the nine years of the study, we did not detect significant annual variation in neonatal SVL, nor any relationship between climatic conditions and SVL. However, longer neonates had more ventral scales. The same correlation between SVL and vertebrate scale number also hold true among the adult females that gave birth ($r = 0.19$, $r^2 = 0.04$, $F(1,132) = 5.41$, $p < 0.02$), confirming a structural link between the two traits.

Hence, our results suggest that thermal regimes experienced during early development directly affected the somitisation process and thus meristic traits (ventral scalation; vertebral number) that are structurally linked to snout-vent length (Fig. 4). Thermal influences on the number of vertebrae have been documented in wide range of taxa including fishes (Fowler 1970), amphibians (Jockusch 1997) and reptiles (Osgood 1978). Our study provides the first field-based empirical demonstration of weather-induced modifications of neonatal phenotypes in a viviparous squamate. Such interactions may directly alter offspring quality and survival. Both laboratory and field studies demonstrate that vertebral number per se may influence fitness-related traits such as size specific growth rate (Arnold 1988, Lindell 1996) and locomotor performance (Arnold 1988, Arnold and Bennett 1988). Hence, the influence of natural climatic conditions on embryo development may affect the quality of offspring produced by a female viper.

Finally, weather conditions affected neonatal fitness directly by influencing rates of embryo mortality. Years with cool weather late in summer, close to the end of gestation (August), resulted in a high incidence of stillborn offspring. This result suggests that embryos may be particularly sensitive to low temperatures late in development, a pattern previously reported in a field study on an oviparous snake species (Burger and Zappalorti 1988). As our study population is close to the species' northern range limit (Stewart 1971), the sensitivity of offspring development to ambient weather conditions may be a direct result of climatic constraints on female thermoregulation. In more favorable environments (e.g. southern populations), viviparity may well allow female asp vipers to selectively alter incubation

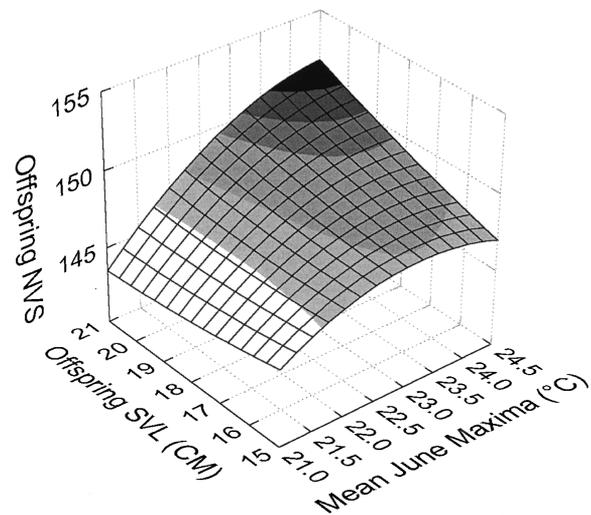


Fig. 4. Influence of mean June ambient thermal maxima ($^{\circ}\text{C}$) on the number of ventral scales (Offspring NVS) and body size (Snout vent length, cm) of neonatal vipers.

conditions and thus provide optimal incubation regimes for their offspring.

The ecological and evolutionary significance of such interactions among the environment, female thermoregulatory behavior, and offspring phenotype is a complex issue that requires further work, notably comparative studies of populations facing different climatic conditions. The high levels of embryo mortality detected in our study probably reflect the location of Les Moutiers at the northern limit of the geographic range of the species. In this area, female aspic vipers experience high survival costs of reproduction and most females reproduce only once in their lifetime (Bonnet et al. 1999b, 2002). Further north the aspic viper is replaced by a sister species, the adder (*Vipera berus*), with limited overlap in the distribution of the two species (Saint Girons 1975). While similar in size and appearance, these two vipers diverge in metabolic rates and in thermal requirements for digestion and gestation. Both are lower in *V. berus* than in *V. aspis*, allowing the former species to penetrate into cooler, more northern areas (Naulleau 1983, 1986, Saint Girons et al. 1985). Our analyses suggest that the thermal optima for embryonic development could also constrain the geographic distribution of *V. aspis* (as suggested for oviparous squamates by Shine 1987 and Shine et al. 2003).

In conclusion, we found that in this northern population of snakes, natural thermal conditions significantly affected embryonic development despite active maternal thermoregulation. Our results underline the importance and complexity of ambient thermal influences on the lives of ectothermic vertebrates. Comparative studies with southern populations facing a less constraining environment would be of great interest. In addition, experimental examination of female thermoregulatory behavior during particular thermosensitive phases (such as early embryogenesis) are needed to clarify to the extent to which viviparity permits active maternal manipulation of offspring phenotypes.

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