Geographic variation of body size and reproductive patterns in Continental versus Mediterranean asp vipers, *Vipera aspis*

MARCO A. L. ZUFFI[1]*, AUGUSTO GENTILLI[2], ELENA CECCHINELLI[1], FABIO PUPIN[2], XAVIER BONNET[3], ERNESTO FILIPPI[4], LUCA M. LUISELLI[4], FILIPPO BARBANERA[5], FERNANDO DINI[5] and MAURO FASOLA[2]

[1]*Museo di Storia Naturale e del Territorio, Università di Pisa, via Roma 79, I-56011 Calci (Pisa), Italy*
[2]*Dipartimento di Biologia Animale, Università di Pavia, p.za Botta 9, I-27100 Pavia, Italy*
[3]*Centre d'Etudes Biologiques de Chizé, CNRS, F-79360 Villiers en Bois, France*
[4]*FIZV (Ecology), via Olona 7, I-00198 Rome, Italy*
[5]*Dipartimento di Biologia (Unità di Protistologia- Zoologia), Università di Pisa, via Volta 6, I-56126 Pisa, Italy*

The occurrence of variation in body size and reproductive traits of *Vipera aspis* was assessed by analysing 74 reproductive females of different populations, collected throughout a large part of the distribution range of the taxon, from central-western France to central Italy. Six populations were analysed, two of plain habitats, in France and Italy, characterized by a Continental climate, whereas the other four derived from two coastal and two inland, hilly Italian habitats, respectively, showing a Mediterranean climate. Females of the French area showed the smallest mean body size, whereas the pre- and post-partum body masses of females from the coolest, central Italic area were significantly higher. Litter size varied among habitats and was significantly correlated with maternal body size. If the snout–vent length feature varied largely among offspring of different habitats (with the longest size occurring in representatives of the coastal, central Italian area), the average of both body mass and total mass of offsprings did not exhibit any clear pattern among populations colonizing different habitats. Nevertheless, relative litter mass was higher in the French continental populations, and lower in the coolest, Mediterranean, Italian ones. It is worth emphasizing the positive correlation between the snout–vent length feature of females and the total litter mass to environmental factors, such as hottest month temperature and total rainfall. The available literature records that female body size affects offspring size and fecundity. If the present study bolsters this correlation, it also rules out any other effect of female body size on the offspring characteristics analysed. Finally, evidence is provided for the role of climatic factors on life-history traits (e.g. pre-partum body mass) of asp vipers, although confounding effects pursued, for example, by food availability may occur. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **96**, 383–391.

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INTRODUCTION

The analysis of intra- and inter-specific variation in life-history traits provides raw material for understanding evolutionary patterns (Seigel & Fitch, 1984;

Iverson *et al.*, 1993; Shine, 2000; Ashton, 2002). Ectotherms are of particular interest because most aspects of their morphology, behaviour, physiology and reproduction are strongly influenced by environmental factors, mainly of climatic nature (Huey & Stevenson, 1979; Angilletta, Niewiarowski & Navas, 2002). In this regard, reptiles are one of the most

*Corresponding author. E-mail: marcoz@museo.unipi.it

studied taxon (Andersson, 2003), particularly as far as their shift from oviparity to viviparity is concerned, fostering reproduction in cold climates (Blackburn, 1982, 1985; Shine, 2002). Most Viperidae opted for such reproductive strategy (Saint Girons, 1992), implemented by storage of large body reserves to face reproductive requirements (Bonnet, Bradshaw & Shine, 1998; Bonnet *et al.*, 2002). A marked reduction or absence of feeding activity during gestation has been interpreted as the outcome of a trade-off between thermoregulation to maintain optimal temperatures for embryos and foraging activity (Lourdais, Bonnet & Doughty, 2002a), which is a strategy most rewarding in pregnant females facing adverse cold conditions. Reduction, up to stopping, of food intake (Pleguezuelos *et al.*, 2007) has strong fitness consequences on litter size, offspring size, post-partum survival, and reproductive frequency (Saint Girons, 1952; Madsen & Shine, 1992; Bonnet *et al.*, 1999, 2002).

During the last four decades, much attention has been focused on the reproductive strategies of Palaearctic viperids and their relationships with other life-history traits (Saint Girons, 1952, 1996; Andrén & Nilson, 1983; Seigel & Fitch, 1984; Madsen & Shine, 1992; Luiselli, 1995; Naulleau & Bonnet, 1996; Bonnet *et al.*, 2002). Nevertheless, the sources and the patterns of variation of reproductive traits along with geographical clines and climatic factors have scarcely been considered. Several studies have investigated reproductive output from single populations, with particular attention to seasonal climatic effects on either juveniles (Lourdais *et al.*, 2004; Altwegg *et al.*, 2005), or the whole, but single population (Madsen & Shine, 1992; Zuffi, Giudici & Ioalè, 1999). Although investigations have been undertaken on reproductive features of populations comprising different subspecies (*Vipera aspis francisciredi* versus *Vipera aspis hugyi*) or species (*Vipera ammodytes*) (Luiselli & Zuffi, 2002), no comparative information has been conveyed about the effects and/or the relationships between climatic factors (e.g. rainfall, average temperature) and reproductive features (e.g. litter size) on different conspecific populations.

The asp viper (*Vipera aspis*) is one of the most morphologically variable snakes of the Palaearctic region (Phisalix, 1968; Brodmann, 1987). Throughout its distribution range, this species has colonized different habitats, which exploits the strict tuning of its activity patterns to seasonal changes (Saint Girons, 1952; Naulleau & Bonnet, 1996; Naulleau, Bonnet & Duret, 1997). The short activity season, the poor body condition of post-parturient females, and the short feeding period after parturition may explain the low breeding frequency of the species at issue, insomuch that, in central-western France, the reproductive rate

of females may be biennial, triennial, or even quadrennial (Saint Girons, 1952, 1957a, b, 1996; Bonnet & Naulleau, 1996; Naulleau & Bonnet, 1996; Bonnet *et al.*, 2002). This notion allows the prediction that activity and feeding seasons would be longer in southern Mediterranean areas where climate is generally warmer. Climatic factors affect growth rate and body size which, in turn, determine most reproductive characteristics; and this kind of relationship is influenced by the positive correlation between maternal size and fecundity recorded by Bonnet *et al.* (2003) in many, but not all viper populations. Hence, any attempt to compare geographic variations of reproductive traits should take body size into account as a key parameter. Central to the present study is the understanding of body size differences among populations colonizing different habitats, and the role of these differences in driving variation of reproductive traits in a predictable way.

MATERIAL AND METHODS

STUDY AREAS

Vipera aspis populations were collected from six localities distributed from central-western France to central Italy: (1) the 'Foret de Chizé' (CHIZ) site was located near Niort (central-western France) (46°05′N, 0°25′W), 55 km East from the Atlantic Coast, and was characterized by an Oceanic climate with Mediterranean influences, accounting for mild winters and hot–dry summers. Vegetation of the site comprised mainly beechs (*Fagus sylvatica*) and oaks (*Quercus* spp.), along with other tree species (e.g. *Carpinus betulus*). The collecting area extended over 2614 ha, at 70 m a.s.l. (2) The 'Riserva Naturale di Bosco della Fontana' (FONT) site was located near Mantua (north-western Italy) (45°12′N, 10°44′E) and represented a relic patch of mesophilic wood (*Quercus robur* and *Carpinus betulus*) surrounding large grassland, with a large central wetland. The collecting area was included into an intensely cultivated landscape. It extended over 230 ha, at 25 m a.s.l. The area's climate was characterized by cold and foggy winters along with warm and sultry summers. (3) The 'Tombolo' (TOMB) site was located at 10 km far from the south-western boundary of Pisa (43°38′N, 10°18′E), at 9 m a.s.l., and 4 km far from Ligurian Sea. It was included into the Natural Park of Migliarino–San Rossore–Massaciuccoli, The site extended over 6 ha of largely sandy land. This area was shared out into cultivated fields bordered by drainage canals which in turn were flanked by reeds (*Phragmites australis*); a wood, comprising *Pinus pinea* and *Quercus ilex*, marked the southern boundary of the site. (4 and 5) 'Tolfa Mountains' sites

Table 1. Average meteorological data for the six study sites (data sets provided by local broadcasting stations)

	CHIZ	FONT	TOMB	TOL1	TOL2	CAST
Overall mean rain fall (mm)	756	658	777	1122	1107	941
Mean temperature of the coldest month (°C)	5.5	1.1	3	4	6	8.9
Mean temperature of the hottest month (°C)	20.4	24.3	26	23	23	26.2

CHIZ, Chizé; FONT, Bosco della Fontana; TOMB, Tombolo; TOL1, Tolfa1; TOL2, Tolfa2; CAST, Castelfusano.

(TOL1, TOL2) were located near Oriolo Romano (central-western Italy), 25 km East from the Tyrrhenian Sea (42°09′N, 12°08′E). TOL1 extended over 100 ha, at 200 m a.s.l., and TOL2 over 70 ha, at 400 m a.s.l. The sites showed similar climatic conditions, exposure, and vegetation (for a detailed description of the area, see Luiselli & Agrimi (1991). This last was represented by bush species of *Spartium* sp., *Paliurus* sp., and *Cytisus* sp., and the woody *Pyrus pyraster* bordered *F. sylvatica* and *Quercus* sp. woodlands. (6) The 'Parco Urbano Pineta di Castelfusano' (CAST) site, located on the Tyrrhenian coast (41°41′N, 12°23′E), 30 km from Rome (central-western Italy), extended over 1000 ha, in the range 0–5 m a.s-l. m. This site was a pinewood (*P. pinea*), bush (*Q. ilex*, *Juniperus oxicedrus*, *Pistacia lentiscus*, *Erica arborea*) and mesophilic wood (*Q. robur*, *Fraxinus excelsior*, *Ulmus minor*) protected area. Meteorological data for each collecting area are shown in Table 1.

SAMPLES COLLECTION AND MEASUREMENTS

A comparatively congrous sample of gravid vipers was considered for each collecting area. During several reproductive seasons (1990–1993, 1995, 2002, and 2004–2006), adult female vipers were captured by hand, sexed by inspecting dorsal coloration features as well as tail morphology, and palpated to determine their reproductive status. Pregnant females were brought to the laboratory, where they were kept until parturition. A total of 74 pregnant specimens from the various sites were analysed: CHIZ, *N* = 10; FONT, *N* = 12; TOMB, *N* = 9; TOL1, *N* = 19; TOL2, *N* = 15; and CAST, *N* = 9. The following parameters were recorded: (1) snout–vent (SVL) and (2) total (TL) length of each female, accuracy ± 0.5 cm; (3) the mass of each female few days (1–2 days) before and (4) immediately after birth (within 2 h), accuracy ± 1 g; (5) the mass and (6) size of the litter from each female; (7) the sex, (8) SVL, (9) TL, and (10) mass of each neonate; and (11) the relative litter mass (RLM), calculated as the ratio between litter mass and maternal post-partum body mass. To avoid any difference due to possible discrepancies in measurements from different researchers, prior to merge our data set, we tested our individual ability and technique in measurement recording, with repeated measures on several specimens. No significant researcher's effect emerged from the analyses (data not shown).

STATISTICAL ANALYSIS

The selected parameters were tested for normality and eventually natural log-transformed in case of need (i.e. SVL, TL). A high degree of correlation among SVL, pre- (female and litter body masses plus embryonic annexes) and post-partum body masses occurred, and we calculated the residuals of these two pairs of parameters from the respective general regressions. Accordingly, we used residuals in all the successive analyses. Moreover, due to the high correlation among most of the considered parameters (i.e. climatic and reproductive features), we plotted all of them into a principal component analysis (PCA) model, using a varimax procedure, pursuing an independent description of the whole biological system. We obtained three principal components explaining 88.6% of the total variance, whose rotated component matrix is shown in Table 2. When the PCA was considered, the first component indicated high positive values for all maternal features and a high negative value for relative clutch mass, the second component indicated high positive values for clutch size and total litter mass, and, finally, the third component indicated high positive values for both individual litter SVL and body masses.

To characterize and/or separate populations representatives of the six sampled areas, the selected parameters were processed with a discriminant analysis, using a stepwise method, selecting the discriminant functions at eigenvalues ≥ 1 and excluding those with tolerance = 0. We also transformed latitude and rainfall (autocorrelated parameters) into a single parameter (thereafter LATRAIN), using PCA (explained variance = 91.0%; component matrix: latitude = −0.954 and rainfall = 0.954). This parameter was used in the successive analyses. A multiple regression analysis was employed to process mean values produced scoring reproductive and meteorological (e.g. average temperatures) parameters in all the six populations analysed. The approach aimed to

Table 2. Varimax rotated component matrix

	Component		
	1	2	3
Natural log SVL	**0.800**	0.457	0.052
Natural log pre-partum body mass	**0.838**	0.492	0.042
Natural log postpartum body mass	**0.896**	0.264	0.111
Natural log litter	0.198	**0.963**	−0.148
Natural log offspring body mass	−0.129	−0.025	**0.866**
Natural log offspring SVL	0.204	0.017	**0.803**
Natural log total litter body mass	0.178	**0.972**	0.117
Arsine relative litter mass	**−0.867**	0.460	0.085

Variables in bold are those contributing the most to component analysis.
SVL, snout–vent length.

Table 3. Body size (SVL) and residuals values (adjusted for SVL) of reproductive females

Locality	SVL	Pre-partum body mass	Post-partum body mass
CHIZ	472.2 ± 31.7 (10)	−0.0306 ± 0.207 (10)	−0.1073 ± 0.174 (10)
FONT	540.5 ± 38.1 (11)	0.064 ± 0.218 (8)	−0.2066 ± 0.195 (11)
TOMB	590.3 ± 21.9 (7)	−0.1418 ± 0.201 (9)	−0.1357 ± 0.137 (8)
TOL1	571.8 ± 51.0 (19)	0.1704 ± 0.144 (17)	0.1911 ± 0.143 (16)
TOL2	554.3 ± 34.0 (15)	−0.106 ± 0.144 (14)	0.0645 ± 0.102 (14)
CAST	550.6 ± 50.1 (9)	−0.0702 ± 0.152 (8)	0.016 ± 0.231 (8)

Data are mean ± SD (N).
CHIZ, Chizé; FONT, Bosco della Fontana; TOMB, Tombolo; TOL1, Tolfa1; TOL2, Tolfa2; CAST, Castelfusano.

disclose parameters affecting reproductive traits. Morover, we followed the same procedure using variances of all the above considered parameters for each locality. Finally, we performed a nested analysis of variance (ANOVA), using R, release 2.5.1 (http://www.r-project.org/), on natural log offspring SVL and body mass, and using individual female viper as a random factor nested in locality. We carried out all the other statistical analyses using SPSS, version 13.0 (SPSS Inc.), with alpha set at 5% and all tests being two-tailed.

RESULTS

COMPARISONS OF MATERNAL AND REPRODUCTIVE TRAITS AMONG POPULATIONS

Female characteristics
Female body size (SVL) differed significantly among the six studied populations (ANOVA, with SVL as dependent variable and locality as factor: $F_{5,65} = 10.471$, $P < 0.001$; Table 3). Post-hoc tests showed highly significant differences between CHIZ and all the other areas (least significant difference, LSD, $P < 0.001$), and between FONT and TOMB (LSD, $P = 0.016$).

Residuals of pre-partum body mass were highly different among populations (ANOVA, with residual pre-partum body mass as dependent variable and locality as factor: $F_{5,57} = 5.908$, $P < 0.001$; Table 3). Post-hoc tests showed highly significant differences between TOL1 and all the other areas (LSD, $P \leq 0.005$), with the exception of FONT. Moreover, FONT differed from TOL2 and TOMB (LSD, $P = 0.037$ and $P = 0.029$, respectively). On average, residuals of post-partum body mass were highly different among populations (ANOVA, with residual post-partum body mass as dependent variable and locality as factor: $F_{5,59} = 10.053$, $P < 0.001$; Table 3). Post-hoc tests showed highly significant differences between TOL1 and all the other areas (LSD, P in the range 0.001–0.037), between TOL2 and all the others with the exception of CAST (LSD, P in the range 0.001–0.037); moreover, CAST differed also from FONT (LSD, $P = 0.005$).

Reproductive output
We found no significant differences in litter size among populations (ANOVA, with litter size as dependent variable and locality as factor: $F_{5,68} = 0.27$,

Table 4. Litter size and offspring features

Locality	Litter size	Average total litter mass	Offspring SVL	Average offspring body mass
CHIZ	6.1 ± 1.4 (10)	36.8 ± 8.0 (10)	178.3 ± 9.2 (10)	6.2 ± 1.1 (10)
FONT	8.3 ± 3.7 (12)	47.9 ± 26.1 (12)	186.0 ± 15.9 (12)	5.7 ± 0.7 (12)
TOMB	7.8 ± 3.7 (9)	48.6 ± 22.7 (9)	202.3 ± 10.4 (7)	6.3 ± 0.5 (9)
TOL1	6.8 ± 2.3 (19)	39.6 ± 13.2 (16)	185.5 ± 8.0 (16)	5.8 ± 0.4 (16)
TOL2	6.9 ± 1.9 (15)	40.8 ± 10.4 (14)	184.5 ± 10.6 (14)	6 ± 0.2 (14)
CAST	7 ± 2.2 (9)	41.6 ± 13.3 (7)	183.2 ± 10.3 (8)	5.8 ± 0.3 (7)

Data are mean ± SD (*N*).
SVL, snout–vent length; CHIZ, Chizé; FONT, Bosco della Fontana; TOMB, Tombolo; TOL1, Tolfa1; TOL2, Tolfa2; CAST, Castelfusano.

Table 5. Relative litter mass

Locality	*N*	Mean	SD
CHIZ	10	32.9	2.10
FONT	8	25.9	4.74
TOMB	9	23.1	6.88
TOL1	16	16.5	4.02
TOL2	14	24.3	3.34
CAST	7	23.4	1.54

CHIZ, Chizé; FONT, Bosco della Fontana; TOMB, Tombolo; TOL1, Tolfa1; TOL2, Tolfa2; CAST, Castelfusano.

$P = 0.928$; Table 4). However, litter size differed significantly (Table 4) when maternal SVL was taken into account, no significant interaction with locality as random factor was observed (ANCOVA, litter size: $F_6 = 5.25$, $P < 0.001$; natlogSVL × locality: $F_5 = 1.891$, $P = 0.108$; natlogSVL: $F_1 = 29.307$, $P < 0.001$). The average total litter mass (Table 4) did not differ significantly among populations (ANOVA, with total litter mass as dependent variable and locality as factor: $F_{5,62} = 0.251$, $P = 0.938$) without further effect of the interaction between residuals post-partum body mass and locality (ANCOVA, total litter mass: $F_6 = 1.066$, $P = 0.393$; respostpartum × locality: $F_5 = 1.249$, $P = 0.299$; respostpartum: $F_1 = 0.052$, $P = 0.82$). Analyses on arcsin transformed RLM highlighted marked differences among populations (ANOVA, with RLM as the dependent variable and locality as factor: $F_{5,58} = 18.855$, $P < 0.001$; Table 5). Post-hoc tests showed that CHIZ had the higher values of RLM, whereas TOL1 had lower values, differing from all the other areas (LSD, $P \leq 0.002$ for both).

Offspring characteristics
Offspring SVL differed significantly (ANOVA, Average litter SVL as dependent variable and locality as factor: $F_{5,61} = 3.875$, $P = 0.004$; Table 4). A nested ANOVA with female identity as a random factor nested in locality and natural log offspring SVL as dependent variable did not show any significant effect. Post-hoc tests showed that TOMB offsprings were significantly longer with respect to all the other areas' newborns (P in the range 0.001–0.004). Average offspring body mass was only marginally different among areas (ANOVA, offspring body mass as dependent variable and locality as factor: $F_{5,62} = 2.031$, $P = 0.087$; Table 4). A nested ANOVA with female identity as a random factor nested in locality and natural log offspring body mass as dependent variable did not show any significant effect. Post-hoc tests showed that average offspring mass of TOMB differed from FONT and TOL1 (LSD multiple comparison test, $P = 0.011$ and $P = 0.028$, respectively), and CHIZ from FONT ($P = 0.037$).

DISCRIMINANT ANALYSIS AND MULTIPLE REGRESSION ANALYSIS

Discriminant analysis found a single main principal function (Eigenvalue = 3.622, 92.9% variance explained, Wilks' lambda = 0.168, $P < 0.001$) that discriminated the populations (Fig. 1), according to maternal and litter parameters. CHIZ and TOL1 appeared as the best characterized, highly homogeneous, populations (group A); FONT, TOL2, and CAST shared the largest amount of total cases (group B); and TOMB (group C) appeared to be intermediate compared with the other groups.

The relationship between each reproductive parameter and the climatic one (e.g. hottest month temperature) has been highlighted using a multiple regression analysis where one reproductive variable was entered in the model each time as the dependent variable and the climatic variables were the covariates. We found marginally significant and positive relationships between average female SVL and
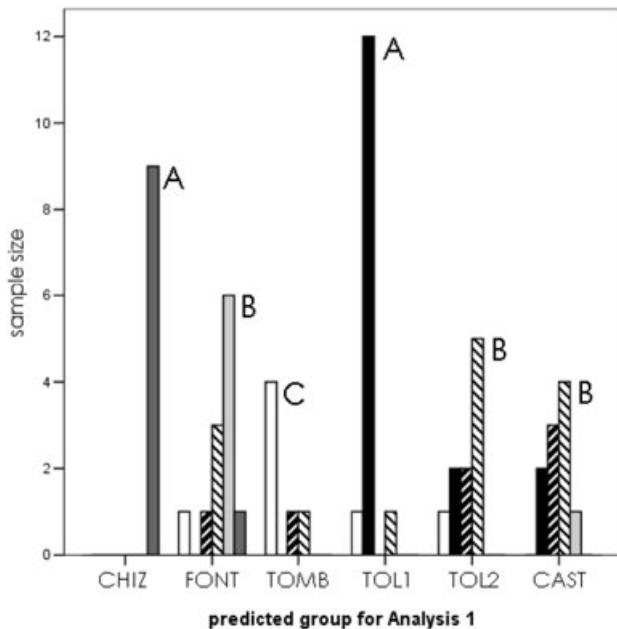
Figure 1. Distribution of study areas and prediction of correct analysis according to selected maternal and litter characteristics in *Vipera aspis* (CHIZ = Chizé, dark grey; FONT = Bosco della Fontana, light grey; TOMB = Tombolo, white; TOL1 = Tolfa1, black; TOL2 = Tolfa2, white + black bars; CAST = Castelfusano, black + white bars). For a description of the various groups, see text.

both the hottest month temperature ($F = 15.84$, d.f. = 1, $P = 0.058$) and LATRAIN ($F = 15.43$, d.f. = 1, $P = 0.059$). Moreover, we found a significant negative relationship between total litter mass and hottest month temperature ($F = 26.01$, d.f. = 1, $P = 0.04$).

MULTIPLE REGRESSION ANALYSIS ON VARIANCES OF SELECTED VARIABLES

We found significant positive relationships between average litter body mass and both hottest month temperature and the variable LATRAIN combining latitude and rainfall (model: $F = 23.85$, d.f. = 3, $P = 0.041$; hottest month temperature: $F = 19.49$, d.f. = 1, $P = 0.048$; LATRAIN: $F = 33.85$, d.f. = 1, $P = 0.028$). We also found a significant relationship between residuals of pre-partum body mass and LATRAIN, while less influence was found in relation to coldest month temperature (model: $F = 173.07$, d.f. = 3, $P = 0.006$; LATRAIN: $F = 287.35$, d.f. = 1, $P = 0.003$; coldest month temperature: $F = 15.36$, d.f. = 1, $P = 0.059$).

DISCUSSION

INTER-POPULATION BODY SIZE DIFFERENCES

A geographic separation between French and Italian asp-viper populations was disclosed. French CHIZ

females were significantly shorter than all representatives of the Italian populations, notwithstanding an intra-group variation among these. Female body size was positively correlated with the hottest month temperature and total rainfall, yet, a reverse correlation occurred with latitude. Accordingly, the available literature records a geographical gradient in body size also in other reptiles (Fornasiero *et al.*, 2007; Sacchi *et al.*, 2007). Bergmann's theory rationalizes the occurrence of intra-specific, large-scale patterns variation in body size, related to environmental factors (Bergmann, 1847; Rensch, 1950; Mayr, 1956). This theory finds its support in taxa such as birds, mammals, and chelonians (James, 1970; Zink & Remsen, 1986; Willemsen & Hailey, 1999; Ashton, Tracy & de Queiroz, 2000; Ashton, 2002; Sacchi *et al.*, 2007), but not as far as lizards and snakes are concerned (Ashton & Feldman, 2003). Our findings are in line with previous findings obtained for other Squamata that reverse Bergmann's theory (Ashton & Feldman, 2003).

BODY SIZE DIFFERENCES AND REPRODUCTIVE FEATURES RELATIONSHIPS

Among all populations analysed, females comprising TOL1 appeared to be the healthiest representatives, versus the largest differences occurring versus the TOMB and TOL2 populations. TOL1 females also displayed a greater pre-partum body mass versus CHIZ, FONT, and TOMB representatives. It should be emphasized that the variance of residuals of pre-partum body mass resulted in an enhancing effect of the higher latitude and lower rainfall on the dispersion of the data (CHIZ, FONT, and TOMB), whereas lower latitude and higher rainfall resulted in inverse effects for the other populations (TOL1, TOL2, and CAST). These results bolster the significant role of rainfall in reducing the variability of the pre-partum body mass, after, for example, food intake and/or thermoregulation opportunities.

It is worth noting that pre-partum maternal mass mixed maternal body reserves with resources already invested into the clutch (= actual mass of the female + mass of the litter) and therefore did not provide any index of maternal fat reserves. However, pre-partum maternal mass reflected the sum of the resources gathered by the female over time, regardless of whether the respective allocation between the mother and the litter is considered. It may be assumed that favourable and warm climatic conditions, associated with a low latitude and higher annual rainfall, favoured foraging activity and prey assimilation, thus contributing to the higher than average overall pre-partum maternal mass. A misleading effect could be produced by food availability,

insomuch as the literature records that food abundance influences vitellogenesis, embryonic development, and reproductive frequency in vipers (Bonnet *et al.*, 2001a; Lourdais *et al.*, 2002a). In this regard, collecting site TOL2 could offer inhabiting vipers a larger opportunity to exploit food resources than that available for females at the TOL1 site (for diversity of prey species at the study area, see Luiselli & Agrimi, 1991; for trapping data, see Filippi, 1995). However, food availability reflects neither food intake by itself, nor food assimilation and energy income, whereas, as stressed above, the interaction between rainfall, sunny hours, basking site availability, and feeding rate can explain different body condition patterns (Bonnet *et al.*, 2000, 2001a; Zuffi *et al.*, 1999).

If natural selection rewards the larger reproductive females with increasing fecundity (Shine & Schwarzkopf, 1992) then we could hypothesize a lower fecundity in central-western France compared with central Italy. Regarding the effect of maternal body size, litter size showed significant differences among all the studied populations. The results obtained suggest that litter size could be more constrained than the other reproductive features and thus selectively stable within the distribution range of *V. aspis* (Bonnet *et al.*, 2002). Geographic differences were recorded in mean maternal body size, and these were large enough to allow the expression of important variation in litter size among populations. In addition, the vipers' reproductive success was not always directly influenced by female body size (e.g. litter size, post partum survival), but it was the result of synergic interactions among favourable food intake, basking, and climatic opportunities (Andrén & Nilson, 1983; Bonnet *et al.*, 1998, 2000; Lourdais *et al.*, 2002b). A marked relationship between female size and reproductive traits among different populations may be thus assumed; and such an assumption is bolstered by our results. Total litter mass did not show any difference among populations, but we observed a significant negative correlation between such a feature: the hottest month temperature and the annual rainfall. This suggests a direct influence of climatic factors on total litter mass. In addition, the variance of average litter body mass was slightly affected by the hottest month temperature. Yet, the total rainfall played a more marked effect on the dispersion of the analysed data (CHIZ, FONT, and TOMB), whereas lower latitude and the higher rainfall negatively influenced the other samples (TOL1, TOL2, and CAST). All of this supports the significant role of rainfall in reducing the variability of the average litter body mass, suggesting a combined effect of food intake and/or thermoregulation opportunities.

RLM varied largely among populations, with central-western France population showing higher values that those of the central Italy. The occurrence of a large intra- and inter-population variability of RLM has already been documented in the asp viper (Bonnet *et al.*, 2003), suggesting that the relative reproductive effort is a trait that responds markedly to local factors (Zuffi *et al.*, 1999). The inter-population differences recorded in the present study were likely due to the continental climate affecting the French population versus the mild climate enjoyed by the Italian ones. Under these latter conditions, a higher than average relative reproductive effort may occur, especially when the survival of reproductive females is particularly low (Bonnet *et al.*, 1998, 1999). RLM is assumed to reflect the trade-off between maternal survival and optimal reproductive output (Seigel & Fitch, 1984; Shine & Schwarzkopf, 1992; Bonnet *et al.*, 2001b, 2002). Slight differences in environmental conditions (i.e. food intake and basking efficiency) may influence the final reproduction balance (Bonnet & Naulleau, 1994). Because the RLM of the French sample was larger than that of the other populations, we propose that this difference is a consequence of a trade-off between mother and newborn survival. Offspring size varied significantly between sites, with TOMB females producing the largest offspring. Yet, the average offspring size did not appear to be significantly affected by the environmental factors. When the average offspring mass was considered, different relationships appeared from inter-population comparisons (e.g. FONT offspring were markedly lighter than those of TOL1 and CHIZ). Nevertheless, such a reproductive feature also was significantly related to an environmental factor. Accordingly, it is no surprise that there is a lack of any significant effect of an individual female viper, nested in locality, as a random effect, and maternal SVL as covariate, on both offspring SVL and offspring body mass.

The three population groups, revealed by discriminant analysis, provide a scenario illustrating a complex reproductive pattern. Vipers living in cooler and semi-continental climatic areas appeared to be similar to each other, whereas, among the Mediterranean populations, TOMB showed additional differences with respect to the other three viper populations, as already noted by Altwegg *et al.* (2005), where climate and life-history traits were shown to interact in similar ways, even across biologically different vertebrate species, thus generalizing such a interactive pattern.

The present study addressed two main issues: if female body size varies geographically, thereby influencing reproduction, it has no evident independent effects on offspring characteristics. Nevertheless,

climatic features (e.g. basking opportunities) play an additive role on some life-history traits (e.g. rainfall versus pre-partum body mass or average offspring body mass), thus interacting with other factors such as food availability.

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REFERENCES

Altwegg R, Dummermuth S, Anholt BR, Flatt T. 2005. Winter weather affects asp viper *Vipera aspis* population dynamics through susceptible juveniles. *Oikos* **110:** 55–66.

Andersson S. 2003. Hibernation, habitat and seasonal activity in the adder, *Vipera berus*, north of the Arctic Circle in Sweden. *Amphibia-Reptilia* **24:** 449–457.

Andrén C, Nilson G. 1983. Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. *Amphibia-Reptilia* **4:** 63–79.

Angilletta MJ Jr, Niewiarowski PH, Navas CA. 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27:** 249–268.

Ashton KG. 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* **11:** 505–523.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: Turtles follow it, lizards and snakes reverse it. *Evolution* **57:** 1151–1163.

Ashton KG, Tracy MC, de Queiroz A. 2000. Is Bergmann's rule valid for mammals? *American Naturalist* **156:** 390–415.

Bergmann C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien* **1:** 595–708.

Blackburn DG. 1982. Evolutionary origins of viviparity in the Reptilia. I. Sauria. *Amphibia-Reptilia* **3:** 185–205.

Blackburn DG. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Icthyosauria. *Amphibia-Reptilia* **6:** 259–291.

Bonnet X, Bradshaw SD, Shine R. 1998. Capital versus income breeding: an ectothermic perspective. *Oikos* **83:** 333–342.

Bonnet X, Lourdais O, Shine R, Naulleau G. 2002. Reproduction in a typical capital breeder: costs, currencies, and complications in the aspic viper. *Ecology (Washington DC)* **83:** 2124–2135.

Bonnet X, Naulleau G. 1994. Utilization d'un indice de condition corporelle (BCI) pour l'étude de la reproduction chez les serpents. *Comptes Rendus de l'Academie de Sciences, Paris, Sciences de la Vie* **317:** 34–41.

Bonnet X, Naulleau G. 1996. Catchability in snakes: consequences for estimates of breeding frequency. *Canadian Journal of Zoology* **74:** 233–239.

Bonnet X, Naulleau G, Shine R, Lourdais O. 1999. What is the appropriate time scale for measuring costs of reproduction in a capital breeder such as the aspic viper? *Evolutionary Ecology* **13:** 485–497.

Bonnet X, Naulleau G, Shine R, Lourdais O. 2000. Reproductive versus ecological advantages to larger body size in female *Vipera aspis*. *Oikos* **89:** 509–518.

Bonnet X, Naulleau G, Shine R, Lourdais O. 2001a. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos* **92:** 297–308.

Bonnet X, Shine R, Lourdais O, Naulleau G. 2003. Measures of reproductive allometry are sensitive to sampling bias. *Functional Ecology* **17:** 39–49.

Bonnet X, Shine R, Naulleau G, Thiburce C. 2001b. Plastic vipers: influence of food intake on the size and shape of Gaboon vipers (*Bitis gabonica*). *Journal of Zoology, London* **255:** 341–351.

Brodmann P. 1987. *Die Giftschlangen Europas und die Gattung* Vipera *in Afrika und Asien*. Bern: Kümmerly-Frey.

Filippi E. 1995. Ecologia di due comunità di serpenti in un'area dell'Italia centrale (Monti della Tolfa, Roma). PhD Thesis, University of Rome, 'La Sapienza'.

Fornasiero S, Corti C, Luiselli L, Zuffi MAL. 2007. Sexual size dimorphism, morphometry and phenotypic variation in the Whip snake *Hierophis viridiflavus* from a central Mediterranean area. *Revue d'Écologie (la Terre et la Vie)* **62:** 73–85.

Huey RB, Stevenson RD. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion on approaches. *American Zoologist* **19:** 357–366.

Iverson JB, Balgooyen CP, Byrd KK, Lyddan KK. 1993. Latitudinal variation in egg clutch size in turtles. *Canadian Journal of Zoology* **71:** 2448–2461.

James FC. 1970. Geographic size variation in birds and its relationship to climate. *Ecology* **51:** 365–390.

Lourdais O, Bonnet X, Doughty P. 2002a. Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). *Journal of Experimental Zoology* **292:** 487–493.

Lourdais O, Bonnet X, Shine R, DeNardo D, Naulleau G, Guillon M. 2002b. Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology* **71:** 470–479.

Lourdais O, Shine R, Bonnet X, Guillon M, Naulleau G. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. *Oikos* **104:** 551–560.

Luiselli L. 1995. The mating strategy of the European adder, *Vipera berus*. *Acta Œcologica* **16:** 375–388.

Luiselli L, Agrimi U. 1991. Composition and variation of diet of *Vipera aspis francisciredi* in relation to age and reproductive stage. *Amphibia-Reptilia* **12:** 137–144.

Luiselli L, Zuffi MAL. 2002. Female life-history traits of the aspic viper (*Vipera aspis*) and sand viper (*V. ammodytes*) from the Mediterranean region. In: Schuett GW, Höggren M, Douglas ME, Greene HW, eds. *Biology of vipers*. Carmel, IN: CPG/Biological Sciences Press, 279–284.

Madsen T, Shine R. 1992. Determinants of reproductive success in female adders, *Vipera berus*. *Oecologia* **92:** 40–47.

Mayr E. 1956. Geographical character gradients and climatic adaptation. *Evolution* **10:** 105–108.

Naulleau G, Bonnet X. 1996. Body condition threshold for breeding in a viviparous snake. *Oecologia* **107:** 301–306.

Naulleau G, Bonnet X, Duret S. 1997. Deplacements et domaines vitaux des femelles reproductrices de viperes aspic *Vipera aspis* (Reptilia, Viperidae) dans le centre ouest de la France. *Bulletin de la Societe Herpetologique de France* **78:** 5–18.

Phisalix M. 1968. La livrée des vipères de France. *Bulletin Museum National Histoire Naturelle, Paris* **4:** 661–676.

Pleguezuelos JM, Santos X, Brito JC, Parellada X, Llorente GA, Fahd S. 2007. Reproductive ecology of *Vipera latastei*, in the Iberian Peninsula: Implications for the conservation of a Mediterranean viper. *Zoology* **110:** 9–19.

Rensch B. 1950. Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. *Bonner Zoologische Beiträge* **1:** 58–69.

Sacchi R, Pupin F, Pellitteri Rosa D, Fasola M. 2007. Bergmann's rule and the Italian Hermann's tortoises: latitudinal variations of size and shape. *Amphibia-Reptilia* **28:** 43–50.

Saint Girons H. 1952. Ecologie et éthologie des Vipères de France. *Annales des Sciences Naturelles, Zoologie, Paris* **14:** 263–343.

Saint Girons H. 1957a. Le cycle sexuel chez *Vipera aspis* (L.) dans l' ouest de la France. *Bulletin Biologique de France et Belgique* **91:** 284–350.

Saint Girons H. 1957b. Croissance et fécondité de *Vipera aspis* (L.). *Vie Milieu* **8:** 265–286.

Saint Girons H. 1992. Strategies reproductices des Viperidae dans les zones temperées fraiches et froides. *Bulletin de la Société zoologique de France* **117:** 267–278.

Saint Girons H. 1996. Structure et évolution d' une petite population de *Vipera aspis* (L.) dans une région de bocage de l' ouest de la France. *Revue d'Ecologie (la Terre e la Vie)* **51:** 223–241.

Seigel RA, Fitch S. 1984. Ecological patterns of relative clutch mass in snakes. *Oecologia (Berl)* **61:** 293–301.

Shine R. 2000. Vertebral number in male and female snakes: the roles of natural, sexual and fecundity selection. *Journal of Evolutionary Biology* **13:** 49–86.

Shine R. 2002. An empirical test of the 'predictability' hypothesis for the evolution of viviparity in reptiles. *Journal of Evolutionary Biology* **15:** 553–560.

Shine R, Schwarzkopf L. 1992. The evolution of reproductive effort in lizards and snakes. *Evolution* **46:** 62–75.

Willemsen RE, Hailey A. 1999. Variation of adult body size of the tortoise *Testudo hermanni* in Greece: proximate and ultimate causes. *Journal of Zoology, London* **248:** 379–396.

Zink RM, Remsen JV Jr. 1986. Evolutionary processes and patterns of geographic variation in birds. In: Johnston RF, ed. *Current ornithology*, Vol. 4. New York, NY: Plenum Press, 1–69.

Zuffi MAL, Giudici F, Ioalè P. 1999. Frequency and effort of reproduction in female *Vipera aspis* from a southern population. *Acta Œcologica* **20:** 633–638.