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Annual Reproduction in Female Adders (*Vipera berus*) from a Montane Environment

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ABSTRACT.—**European vipers are typical capital breeders and most studies have revealed that females usually reproduce once every two or three years and that, in some cases, females reproduce once during their lifetime. This paper presents evidence, based on monitoring marked snakes in consecutive years, that several female adders (*Vipera berus*) from a montane environment from the Northern Romanian Carpathians reproduced annually (2007–2008), and also gives preliminary data on other female reproductive traits. Reproductive females and their offspring were smaller and lighter than *V. berus* individuals from most previously studied populations. Maternal traits were not significantly correlated with litter and offspring characteristics.**

Variation in life-history traits offers raw material for natural selection and represents a central theme in evolutionary ecology (e.g., Lourdais et al., 2002). The reproductive strategies of snakes have received great attention during the past three decades, both by evolutionary ecologists and conservation biologists (e.g., Shine and Bonnet, 2009). Viviparous snakes are capital breeders and present an obvious temporal separation between the phase of energy acquisition and investment in reproduction as opposed to income breeders (e.g., a number of oviparous snakes), which acquire and expend the resources necessary for reproduction simultaneously (e.g., Bonnet et al., 1999; Lourdais et al., 2002).

European vipers are typical capital breeders and their reproductive biology has been studied in detail during the past two decades. *Vipera aspis* (e.g., Saint-Girons, 1957; Bonnet et al., 1999; Zuffi et al., 1999; Lourdais et al., 2002; Zuffi et al., 2009) and *Vipera berus* (e.g., Saint-Girons and Kramer, 1963; Nilson, 1981; Andrén and Nilson, 1983; Luiselli, 1992; Madsen and Shine, 1992, 1993; Capula and Luiselli, 1994; Monney et al., 1995) are the most commonly used model organisms among European snakes. The majority of the above-mentioned studies have shown that, in European vipers, capital breeding is linked strongly to a biennial or triennial reproductive cycle in females.

The adder (*V. berus*) is a widespread, Eurasian, small-sized (usually <80 cm total length), venomous snake, encountered in a wide variety of landscapes throughout its range (e.g., Mallow et al., 2003). In Romania, the species is widespread throughout the Carpathian Mountains (*Vipera berus berus*) and is also present in the forest-steppe zones of eastern Romania (*Vipera berus nikolskii*; Zinenko et al., 2010). It is considered an endangered species on a national level (Iftime, 2005). Although *V. berus* is regarded as the world's most studied snake species from an ecological perspective, previous detailed studies on its reproductive biology are strongly focused toward Swedish and alpine populations (e.g., Andrén and Nilson, 1981; Luiselli, 1992; Madsen and Shine, 1992, 1993, 1994; Capula and Luiselli, 1994; Monney et al., 1995).

We present evidence for an annual reproductive frequency in *V. berus* females and preliminary data on other aspects of the reproductive biology from a population in the northern Romanian Carpathian Mountains. To our knowledge, this is the first study on the reproductive characteristics of *V. berus* from the Carpathian Mountains.

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MATERIALS AND METHODS

The study area is located in the northern group of the eastern Romanian Carpathian Mountains. (47°32′23″N; 25°34′46″E). The average annual temperature in the area is around 6.4°C; the average temperature in July is 16.5°C, whereas in January, temperatures drop to an average of −4.9°C (Tufescu et al., 1995). A detailed description of the study site is given in Strugariu and Zamfirescu (2011).

This *V. berus* population is the subject of an ongoing field study conducted by the first author, which began in 2002 but was mainly aimed at studying activity patterns and habitat selection until the year 2007. All captured *V. berus* adults were marked by a unique combination of ventral scale clippings and photographed in detail for further identification. The reproductive status of females was determined through palpation of the ventrum. Between 8 and 11 August 2008, seven gravid *V. berus* females were taken into captivity and kept in individual plastic terraria until after parturition. Water was available ad libitum and food was offered in the form of young laboratory mice (however, none of the females accepted prey during captivity). Mass was determined for these *V. berus* females as well as their neonates (body mass, BM) with a digital scale to the nearest 0.1 g. Mass was determined in adult females before (pre-partum BM) and after parturition (post-partum BM) and measured for snout–vent length (SVL) and total length (TL) with a measuring tape to the nearest millimeters. The total litter mass (TLM) was also recorded as well as the relative litter mass (RLM), which was calculated as the ratio between total litter mass and female post-partum body mass (e.g., Zuffi et al., 2009). *Vipera berus* females and their neonates were released two weeks after parturition at the exact place of capture. With the exception of RLM, all measurements used in parametric correlation analyses were natural log transformed. However, post-partum BM presented a nonnormal distribution even after transformation; consequently, a nonparametric correlation (Spearman's correlation) was used in that case. Pearson correlation was used unless otherwise stated. Parametric conditions were checked prior to using parametric tests, and all data presented in the text are given as mean ± 1 SD. XLSTAT-Pro for Windows was used for all statistical procedures.

RESULTS

Eight adult *V. berus* females were captured during August 2008. Of these, seven females were gravid. Four of the seven gravid females as well as the sole nonreproductive female were individuals that were first captured and marked in July and

August 2007 and were gravid at that time. The other three gravid females captured in 2008 were previously unknown snakes; hence, it is unknown whether they were reproductive during the previous year. Parturition took place between 22 August and 13 September. One of the females that reproduced in consecutive years delivered six stillborn neonates and died immediately after parturition, but the exact cause of death was unknown.

Descriptive statistics for maternal SVL, TL, pre- and post-partum BM, litter size, TLM, RLM, offspring SVL, TL, and BM of the studied population are shown in Table 1 together with a brief literature review of reproductive characteristics of *V. berus* from other regions in Europe. Female neonates (with a mean SVL of 14.2 ± 0.66 cm; $N = 23$) were longer than the males (13.8 ± 0.79 cm; $N = 20$), but the difference was not statistically significant (*t*-test: $t_{41} = 1.71$, $P = 0.095$). Female neonates (2.83 ± 0.24 g; $N = 23$) were slightly heavier than were males (2.77 ± 0.3 g; $N = 20$), but again this was not significantly different (*t* test: $t_{41} = 0.77$, $P = 0.445$).

Litter size was not significantly correlated to maternal SVL ($r = 0.09$, $P = 0.49$, $N = 7$), pre-partum BM ($r = 0.01$, $P = 0.838$, $N = 7$), or post-partum BM (Spearman correlation: $r = 0.14$, $P = 0.45$, $N = 6$). No significant relationships were revealed between maternal SVL and RLM ($r = 0.42$, $P = 0.158$, $N = 6$), TLM ($r = 0.05$, $P = 0.67$, $N = 6$), neonate SVL ($r = 0.07$, $P = 0.59$, $N = 6$), or neonate BM ($r = 0.05$, $P = 0.67$, $N = 6$).

Discussion

A comprehensive set of empirical evidence has shown that females from most European viper populations present a biennial or even triennial reproductive cycle (e.g., *V. berus:* Prestt, 1971; Andrén and Nilson, 1983; Madsen and Shine, 1992; *Vipera ursinii:* Baron, 1997; *V. aspis:* Bonnet et al., 1999; *Vipera latastei:* Pleguezuelos et al., 2007). Female adders are highly sedentary and do not usually feed during pregnancy except for years with very high prey abundance and, thus, are usually emaciated following parturition (e.g., Madsen and Shine, 1992). The cost of reproduction for female vipers seems to be so high that in some populations many specimens reproduce only once in their lifetime (e.g., Madsen and Shine, 1992; Bonnet et al., 2002). However, very rare cases of annual reproduction in *V. aspis* and *V. berus* have been reported previously (Madsen and Shine, 1992; Capula and Luiselli, 1994; Zuffi et al., 1999; Völkl and Thiesmeier, 2002; Nilson et al., 2005). Zuffi et al. (1999) have shown that at least one central Italian population of asp viper (*V. aspis*), living in a warm Mediterranean climate, presents an annual reproductive cycle. In this case, the ability of *V. aspis* to reproduce annually has been attributed to the warmer coastal climate, which offers vipers a prolonged post-partum period for foraging during autumn and, thus, for gathering sufficient reserves to sustain another pregnancy in the following year (Zuffi et al., 1999). In southern Sweden, Madsen and Shine (1992) observed only one female *V. berus* that reproduced in two consecutive years during a period of very high prey abundance. Also, Capula and Luiselli (1994) have shown that, in a color–dimorphic, alpine *V. berus* population, some melanistic females reproduced in consecutive years, whereas all zigzag specimens reproduced once every two-three years. In this case, the increased frequency of reproduction of melanistic females was attributed to the higher thermal efficiency generally associated with melanistic snakes (e.g., Gibson and Falls, 1979), which allows them to be active and forage during colder days in which zigzag vipers are not active; thus, melanistic snakes can accumulate energy reserves necessary for reproduction at a much faster rate. Although Romania lies at the southern limit of the range of the nominate subspecies of *V. berus*, the average climatic characteristics of our study area are probably not the main factors responsible for the observed annual reproductive cycle. The area is one of the coldest places in Romania (5.8–6.6°C average annual temperatures; 13.88–15.14°C average summer temperatures: http://www.tutiempo.net/en/Climate/CIMPULUNG_MOLDOVENE/150400.htm), presenting lower annual average temperatures and similar summer (May through September) temperatures to one of the Swedish habitats (6.7–8.8°C average annual temperatures; 13.18–15.2°C average summer temperatures: http://www.tutiempo.net/en/Climate/SMYGEHUK_LGT-H/26380.htm) where the reproductive biology of *V. berus* was studied (e.g., Madsen and Shine, 1992). However, we speculate that the unusual weather conditions of the year 2007 might be responsible for our observations. The summer of 2007 was among the hottest and driest years in Romania from 1901 to 2007, second only to the year 1946 (Busuioc et al., 2007). During that summer, viviparous snakes (*V. b. nikolskii, Vipera ursinii moldavica*, and *Coronella austriaca*) from populations from lowland eastern Romania, which usually give birth in August or September, did so before the end of July (Strugariu et al., 2011). Females from the montane *V. berus* population discussed in this paper did not appear to reach parturition earlier than usual in 2007 (i.e., compared to previous "normal" years as well as 2008). However, because the *V. berus* was still gravid in August, it is possible that the warmer than usual conditions during that autumn allowed for a prolonged post-partum feeding activity prior to hibernation and the acquisition of sufficient reserves to reproduce again the following year. Another possible explanatory factor for the observed consecutive breeding years could be that prey abundance was high during 2007. Long-term study of the temporal variation in reproductive characteristics in this population is needed to test these potential scenarios and investigate whether the observed annual reproductive cycle is a common occurrence or the result of a rare and unusually warm summer. Such long-term research, coupled with experimental studies, could also shed light on the effects of global climate warming on the reproductive frequency of temperate region viviparous snakes.

Vipera berus is known to be sexually dimorphic with regard to body size (Mallow et al., 2003), and significant differences have been observed between body lengths of males and females in adults from the study population (Strugariu and Zamfirescu, 2011). The absence of intersexual differences in body size and mass in neonate *V. berus* could indicate that the pattern of sexual size dimorphism (SSD) in this species resembles that of *V. ursinii*, where the degree of SSD increases ontogenetically (Tomović et al., 2010). Nevertheless, future detailed research on this topic is needed to verify this assumption.

The average total length of reproductive females from the northern Romanian Carpathians was generally similar to the Italian (Capula and Luiselli, 1994) and Swiss (Monney et al., 1995) populations (Table 1). All the other litter and neonate traits except litter size and RLM displayed smaller values than the other *V. berus* European populations (Table 1). This situation may be the result of a low food resource or to the cost of annual reproduction. Alternately, the small offspring BM could potentially represent an enabling factor for annual breeding because smaller neonates normally take less time to develop.

TABLE 1. Descriptive statistics of reproductive variables (see the Materials and Methods section for abbreviations) in Romania (this study) and comparison with data from other European *Vipera berus* populations (literature data).

Variable (*N*)/Region	Romania (This study); mean ± SD (min–max)	Sweden (Madsen and Shine, 1992); min and max of mean ± SD in 4 years	Sweden (Andren and Nilson, 1983)		Italy (Capula and Luiselli, 1994); mean ± SD (min–max)	Switzerland (Monney et al., 1995); mean ± SD or SE (not specified)	France (Saint-Girons and Naulleau, 1981); mean ± SD
			Low food resource year	High food resource year			
Maternal SVL (cm; 7)	47.45 ± 1.72 (45–50)	51.1 ± 3.1 – 53.8 ± 3.3	–	–	–	–	–
Maternal TL (cm; 7)	52.82 ± 2.26 (50.2–57)	–	67.1 ± 3.83 (61–75)	66.5 ± 6.19 (54–73)	54.29 ± 6.82 (48–67.5)	52 (extracted from chart)	62.21 ± 3.88
Maternal pre-partum BM (g; 7)	89.04 ± 1.71 (81.4–96)	–	–	–	–	–	–
Maternal post-partum BM (g; 6)	43.54 ± 2.89 (38.4–45.7)	–	–	–	–	–	76.14 ± 24.13
Litter size (7)	7.57 ± 1.71 (6–11)	4.7 ± 2.4 – 7.9 ± 3.2	7.5 ± 2.2 (4–13)	10.4 ± 2.7 (6–15)	6.04 ± 2.7 (3–12)	6.7 ± 1.8	7.06 ± 2.78
TLM (g; 6)	20.12 ± 5.33 (11.2–27.5)	–	36.6 ± 10.6 (22.9–62.4)	56.3 ± 17.1 (22–82.8)	–	–	34.04 ± 14.89
RLM (g; 6)	0.46 ± 0.13 (0.24–0.65)	0.33 ± 0.11 – 0.48 ± 0.16	0.241 ± 0.087 (0.128–0.466)	0.272 ± 0.031 (0.21–0.326)	–	–	0.456 ± 0.166
Neonate SVL (cm; 43)	14.03 ± 0.72 (12.5–15.2)	15.3 ± 0.9	–	–	–	–	–
Neonate TL (cm; 43)	15.9 ± 0.75 (14.4–17.1)	–	19.5 ± 1.01 (17.5–21.5)	19 ± 1.17 (16.8–20.7)	–	16.7 ± 0.9	18.74 ± 1.73
Neonate BM (g; 43)	2.8 ± 0.27 (2.2–3.3)	3.95 ± 0.59	4.9 ± 0.62 (4.1–6.2)	5.4 ± 1.2 (3.7–7.7)	–	3.4 ± 0.4	4.73 ± 0.77

Consequently, reproductive females would be able to shorten their gestation period and prolong their pre- and post-gestational foraging period. North American pit-viper species that frequently reproduce annually also tend to have smaller neonates than species that breed less frequently (e.g., *Crotalus pricei*, Prival and Schroff, 2008; *Sistrurus miliarius*, Farrell et al., 2008).

The lack of significant correlations between maternal (body size and mass) and offspring traits (average offspring size and mass, TLM) observed in our population appears to be unusual in some *V. berus* populations (e.g., Madsen and Shine, 1992; Capula and Luiselli, 1994; Monney et al., 1995); consequently, it is probably a result of our small sample. Nevertheless, this situation has been observed in at least one population of *V. berus* and one population of *V. aspis* during periods of low food availability (Andrén and Nilson, 1983; Lourdais et al., 2002).

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Literature Cited

Andrén, C., and G. Nilson. 1981. Reproductive success and risk of predation in normal and melanistic color morphs of the adder, *Vipera berus*. Biological Journal of the Linnean Society 15:235–246.

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

Baron, J. P. 1997. Demographie et dynamique d'une population francaise de *Vipera ursinii* (Bonaparte, 1835), Ecole Pratique des Hautes Etudes, France.

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

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

Busuioc, A., A. Dumitrescu, E. Soare, and A. Orzan. 2007. Summer anomalies in 2007 in the context of extremely hot and dry summers in Romania. Romanian Journal of Meteorology 9:1–17.

Capula, M., and L. Luiselli. 1994. Reproductive strategies in alpine adders, *Vipera berus*—the black females bear more often. Acta Oecologica-International Journal of Ecology 15:207–214.

Farrell, B. D., M. A. Pilgrim, and P. G. May. 2008. Annual variation in neonate recruitment in a Florida population of the pygmy rattlesnake, *Sistrurus miliarius*. *In* W. K. Hayes, K. R. Beaman, M. D. Cardwell, and S. P. Buch (eds.), The Biology of Rattlesnakes, pp. 257–264. Loma Linda University Press, California.

Gibson, A. R., and J. B. Falls. 1979. Thermal biology of the common garter snake *Thamnophis sirtalis* (L). 1. Temporal variation, environmental-effects and sex-differences. Oecologia 43:79–97.

Iftime, A. 2005. Reptile. *Vipera berus*. *In* N. Botnariuc and V. Tatole (eds.), Cartea rosie a vertebratelor din România, p. 189. Muzeul National de Istorie Naturala Grigore Antipa, Bucarest.

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

Luiselli, L. 1992. Reproductive success in melanistic adders—a new hypothesis and some considerations on Andren and Nilson (1981) suggestions. Oikos 64:601–604.

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

———. 1993. Costs of reproduction in a population of European adders. Oecologia 94:488–495.

———. 1994. Components of lifetime reproductive success in adders, *Vipera berus*. Journal of Animal Ecology 63:561–568.

Mallow, D., D. Ludwig, and G. Nilson. 2003. True vipers: natural history and toxinology of old world vipers. Krieger Publishing Co., Malabar, FL.

Monney, J.-C., L. Luiselli, and M. Capula. 1995. Correlates of melanism in a population of adders (*Vipera berus*) from the Swiss Alps and comparisons with other alpine populations. Amphibia–Reptilia 16: 323–330.

Nilson, G. 1981. Ovarian cycle and reproductive dynamics in the female adder, *Vipera berus* (Reptilia, Viperidae). Amphibia–Reptilia 2:63–82.

Nilson, G., C. Andrén, and W. Völkl. 2005. *Vipera (Pelias) berus* (Linnaeus, 1758)—Kreuzotter. *In* U. Joger and N. Stumpel (eds.), Handbuch der Reptilien und Amphibien Europas, 3/IIB Schlangen (Serpentes). III Viperidae, pp. 213–292. Aula Verlag, Wiesbaden.

Pleguezuelos, J. M., X. Santos, J. C. Brito, X. Parellada, G. A. Llorente, and S. Fahd. 2007. Reproductive ecology of *Vipera latastei*, in the Iberian Peninsula: implications for the conservation of a Mediterranean viper. Zoology 110:9–19.

Prestt, I. 1971. An ecological study of the viper *Vipera berus* in southern Britain. Journal of Zoology 164:373–418.

Prival, D. B., and M. J. Schroff. 2012. A 13-year study of a northern population of twin-spotted rattlesnakes (*Crotalus pricei*): growth, reproduction, survival, and conservation. Herpetological Monographs 26:1–18.

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

Saint-Girons, H., and E. Kramer. 1963. Le cycle sexuel chez *Vipera berus* (L.) en montagne. Revue Suisse De Zoologie 70:191–221.

Saint-Girons, H., and G. Naulleau. 1981. Poids des nouveaunes et strategies reproductrices des viperes Europeennes. Terre Vie 35:597–616.

Shine, R., and X. Bonnet. 2009. Reproductive biology, population viability and options for field management. *In* S. J. Mullin and R. A. Seigel (eds.), Snakes: Ecology and Conservation. Comstock Publishing Co. Associates/Cornell University Press, Ithaca, NY.

Strugariu, A., and S. R. Zamfirescu. 2011. Population characteristics of the adder (*Vipera berus berus*) in the northern Romanian Carpathians with emphasis on colour polymorphism: is melanism always adaptive in vipers? Animal Biology 61:457–468.

Strugariu, A., S. R. Zamfirescu, I. Gherghel, and T. C. Sahlean. 2011. Unusual early parturition in temperate region viviparous snakes during the atypically hot summer of 2007. Herpetologica Romanica 5:43–49.

Tomović, L. M., J. M. Crnobrnja-Isailovic, R. D. Ajtic, I. D. Aleksic, and S. Z. Djordjevic. 2010. When do meadow vipers (*Vipera ursinii*) become sexually dimorphic? ontogenetic patterns of sexual size dimorphisms. Journal of Zoological Systematics and Evolutionary Research 48:279–282.

Tufescu, V., C. Giurcăneanu, and I. Mierlă. 1995. Geografia României. Editura Didactică şi Pedagogică, Bucharest.

Völkl, W., and B. Thiesmeier. 2002. Die Kreuzotter. Ein Leben in festen Bahnen? Laurenti Verlag, Bielefeld, Germany.

Zinenko, O., V. Turcanu, and A. Strugariu. 2010. Distribution and morphological variation of *Vipera berus nikolskii* Vedmederja, Grubant et Rudaeva, 1986 in western Ukraine, Republic of Moldova and Romania. Amphibia–Reptilia 31:51–67.

Zuffi, M. A. L., F. Giudici, and P. Ioale. 1999. Frequency and effort of reproduction in female *Vipera aspis* from a southern population. Acta Oecologica-International Journal of Ecology 20:633–638.

Zuffi, M. A. L., A. Gentilli, E. Cecchinelli, F. Pupin, X. Bonnet, E. Filippi, L. M. Luiselli, F. Barbanera, F. Dini, and M. Fasola. 2009. Geographic variation of body size and reproductive patterns in Continental versus Mediterranean asp vipers, *Vipera aspis*. Biological Journal of the Linnean Society 96:383–391.

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