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Reproductive biology of levant viper (Serpentes: *Macrovipera lebetina obtusa* dwigubsky, 1832)

Iskenderov TM
Institute of Zology, Azerbaijan National Academy of Sciences, Baku, A.Abasov Street, Azerbaijan

Abstract
In the paper adaptive significance of a confinement of the oviposition of levant viper, *Macrovipera lebetina obtusa* (Dwigubsky, 1832) to certain embryogenesis stages is considered. It is experimentally proved that the embryos of levant viper have the highest resistance to temperature influences at the time of oviposition and they are capable to survive severe high and low temperatures. In different populations the thermal tolerance of embryos at the stage of oviposition was similar. There is no pronounced intraspecific morphological variability in development of embryos of levant viper by the time of oviposition.

Keywords: oviparous reptiles; levant viper; reproduction; adaptive; embryonic development

Introduction
The variety of strategies for the reproduction of the first terrestrial vertebrates - reptiles - is the result of their evolutionary adaptations to the earthly mode of life. In the process of evolution, various adaptive forms and features emerged in the reproductive activity of reptiles, which allowed their wider distribution on land. The common achievement of reptiles for land development and being able to breed outside the aquatic environment was the acquisition by them of a special type of egg. These eggs had inside all the necessary structural and organizational elements and material resources for the autonomous development of the embryo until the hatching of a new generation. The appearance of a new type of egg was made possible by the biological progress of reptiles along the path of amorphosis (structural and morphophysiological changes), which allowed them to establish new relationships with the external environment [1]. When choosing the fate of the eggs in which the embryo develops, different representatives of reptiles behaved differently in the process of evolution. Taking into account the peculiarities of the structure of eggs and the level of protection of the developing embryo, as well as the conditions of the habitat, evolution predetermined various reproductive strategies for various reptilian representatives, and they became oviparous, egg-viviparous, or viviparous.
For oviparous species of reptiles the strict confinement of the oviposition to the certain developmental stage is typical. In turtles laid eggs contain early embryos on 3-4 day of development [2, 3], while in snakes embryos are developing 33-35 day at the moment of egg-laying, [4, 5]. Unfortunately, the works dealing with experimental studying of adaptive features of reproduction strategies of reptiles are scarce. Therefore, I have studied the morphological variability of embryos at the time of egg-laying in different populations of levant viper (both lowland to mountain areas) and adaptive significance of a confinement of their egg-laying to certain stages of development.

Material and Methods
The material was collected in 2006 in Experimental Stationary of Scientific-Research Center "Zootoxins" of the Ministry of Health of the Azerbaijan Republic. Pregnant females of levant viper *Macrovipera lebetina obtusa* (23 individuals) were caught during a reproduction season (May-June) in different altitude belts (lowland – Imishly district, low mountain – Akhsu district and mountain – Khyzy district). The captured females were reared in the enclosures located in the territories of their natural habitats (group A – lowland; group B – low mountain area, group C – mountain area). The pregnant females of levant viper laid eggs in specially prepared shelters. In total, 169 eggs were laid. For the purpose of determination of development stages of embryos at the time of oviposition 2 eggs were opened in each clutch. Determination of embryonic stages was made according to the table of embryonic development of levant viper [5]. After collection of their eggs all snakes were released back to their natural habitats.
For the studying of temperature stability of embryos of different stages (before, at the time, and after egg-laying) the eggs of snakes were exposed to different temperature influences. To check the thermoresistance of embryos before oviposition, the pregnant females were reared during 5 days at temperature of +15-18^{0}C (hypothermia) or during 2 hours at temperature of +36-38^{0}C (hyperthermia). Later, the eggs were incubated under normal conditions up to 8 days and the numbers of the survived eggs (embryos) were counted. To check the thermal stability of embryos at the stage of oviposition newly laid eggs were exposed to temperature influences. To establish the thermoresistance of embryos of late stages (after oviposition) the eggs were incubated under normal conditions during 2 days and then treated the same way as second group. The eggs of levant viper were incubated according to the method developed by author [5].

Results and Discussion
Thermoresistance of embryos. Experiments have shown that

temperature stability of embryos of levant viper before, after and at the time of egg-laying are different. The highest resistance to the influence of hypo- and hyperthermia was observed in embryos at the time of oviposition, i.e. by the day of egg-laying embryos reach maximum ability to survive adverse temperature effects of environment. In all experiments freshly layid eggs demonstrated high thermoresistance and percentage of survival was 85.7-100% in spite of temperature treatment (*Table*). As it is seen from the Table 1 the similar thermal stability exhibited 2-day embryos. However, the embryos which were in the oviducts of females are highly sensitive to hypo- and hyperthermia and their survival rate constituted 0 – 14.3% (*Table*). At the same time, the comparison of the results of hypo - and hyperthermia indicates that embryos are more sensitive to high temperatures, than to hypothermia. It was observed both in newly-laid eggs and eggs after two days of incubation that the percentage of survival decreased from 100 to 85.7% or even more. In addition to the direct influence on embryos, hyper thermal (36^0C - 38^0C) conditions could also influences the state of substratum used for egg incubation.

Thus, in levant viper snake the eggs are laid when embryos reach the maximal resistance to temperature fluctuations during their development. The confinement of egg-laying to these stages of development of embryos has adaptive significance, because at these stages embryos are capable to survive severe temperature influences of the environment. Comparison of data obtained from different groups shows that in all populations, inhabiting various landscape altitudes, embryos demonstrate similar resistance to thermal influences of environment.

Morphological variability of embryos at the time of oviposition. We determined the stages of development of embryos at the time of oviposition in levant vipers from different populations. Also intraspecific morphological variability of embryos by the time of egg-laying in different populations was studied.

Unlike turtles, the embryonic period of snakes ends, when the eggs are in a genital tract of females [4, 5, 6, 7]. We have established that in levant viper the egg-laying is confined to late stages of embryo development, i.e. initial stages of prenatal period [5, 8]. At the time of oviposition, levant viper embryos are at a completion stage of formation of the mandible and the beginning of tongue formation (*figure*). These stages correspond to 33-35 day age of embryos [5], i.e. at the moment of oviposition the females of levant viper bear the embryos within 33-35 days, to the stage of development which has maximal resistance to temperature influences of environment.

While comparing morphological features of the embryos observed in different populations (lowland, submountain and mountain) of levant viper, we have revealed some variations in the extent of development of the mandible and curls of a tail part by the day of egg-laying. However the low level of these differences does not result in more noticeable morphological variability.

The egg-laying is restricted to narrow range of degree of embryonic development which is characterized by high level of resistance to the temperature fluctuation. Such adaptive feature of reproduction allows a levant viper to breed successfully at various altitudes within its range, from plains to mountain steppes up to the height of 800 - 1200 m a.s.l.

At the same time, comparison of embryonic development of *Macrovipera lebetina obtusa* and that of a water snake *Natrix tessellata* described in literature [4] has allowed to reveal some differences in the degree of development of embryos by the time of oviposition between two species of snakes. By the day of egg-laying the embryos of the levant viper already have a tongue in the form of a swelling, whereas in embryos of water snake it appears only at the 5th day of incubation (*figure*).

Fig 1: Appearance of embryos of the Levanty gyurza at the time of egtg-laying

Table 1: The rate of survival of embryos after 8-day incubation, after temperature influences (hypo - and hyperthermia) in different populations of a gyurza, Macrovipera lebetina

Snake populations according to habitats, number of females and eggs	Temperature Influences, Hypothermia: +15-18^0 C, Hyperthermia: +36-38^0 C	Number of eggs, n (opened eggs)	Survival of embryos in %		
			At the time of egg-laying, n = number of eggs	Before egg-laying (early embryos), n = number of eggs	After egg-laying (2-day embryos), n = number of eggs
Group A - lowland, number ♀♀: 10 number egg: 68	Hypothermia	36 (6)	(n= 10) 100,0 %	(n= 10) 10,0 %	(n= 10) 90,0 %
	Hyperthermia	32 (4)	(n=9) 88,8 %	(n= 9) 0 %	(n= 10) 90,0 %
Group – B submountain, number ♀♀: 7 number eggs: 49	Hypothermia	25 (4)	(n=8) 100,0 %	(n=8) 12,5 %	(n=5) 100,0 %
	Hyperthermia	24 (4)	(n=5) 80,0 %	(n=8) 0 %	(n=7) 11,5 %
Group – C Mountain number ♀♀: 9 number eggs: 52	Hypothermia	27 (4)	(n=8) 100,0 %	(n=8) 14,3 %	(n=7) 100,0 %
	Hyperthermia	25 (4)	(n=7) 85,7 %	(n=7) 14,3 %	(n=7) 85,7 %

Conclusion

The levant viper is an oviparous species occurring in semi-deserts and arid steppes as well as xerophytes mountain areas [9, 10]. The strategy of reproduction in levant viper has developed as a result of adaptations to these habitats [11]. Investigation has shown that females bear and lay eggs at

more late and most heat-resistant stages of morphogenesis of embryos. After oviposition, during natural incubation of eggs, the embryos at these stages can successfully survive adverse effects of high or low temperature factors. Thereby, the present work experimentally proves adaptive significance of a confinement of egg-laying to late stages of embryonic development in levant viper, and this feature of reproduction may have developed as species adaptation. The confinement of egg-laying to certain stages of development of embryos also helps to understand the ways of evolution of reproductive strategies in oviparous snakes which, apparently, went on the way of the choice of the most resistant stages of embryonic development for egg-laying up to ovoviviparity.

Because of wide geographical distribution of levant viper, the morphological variability of embryos by the day of oviposition in different populations has been investigated. It is established that there is no pronounced intraspecific morphological variability in development of levant viper embryos at the time of egg-laying. The following conclusions can be made:

1. In levant viper (*Macrovipera lebetina obtusa*) the egg-laying is confined to initial stages of prenatal period of development of embryos, i.e. stages when the formation of the mandible is completed and formation of tongue begins.
2. It is experimentally proved that by the day of egg-laying the embryos of levant viper (*Macrovipera lebetina obtusa*) reach the greatest resistance to temperature influences of the environment. It is the main adaptive significance of confinement of egg-laying to certain stages of development of embryos. In different populations the thermoresistance of embryos at stage of oviposition was similar.
3. There is small morphological variability of embryos from different populations of *Macrovipera lebetina obtusa* at the day of oviposition. Some variations in extent of development of mandible and curls of a tail part of embryos are revealed. However, the low level of these changes does not result in more noticeable morphological variability by the time of oviposition.

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