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Eggs features and tactical variability in the reproduction of different egg-laying species of reptiles (Testudines, Ophidia) in Azerbaijan

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Abstract

The article discusses the features of eggs, the confinement of oviposition to certain stages of embryonic development, and its adaptive significance in some oviparous reptile species in Azerbaijan. Information is reported on the size and numbers of eggs in clutches, stages of development of embryos at the time of oviposition, as well as the timing of natural incubation of eggs in the Mediterranean tortoise (*Testudo graeca ibera*) and the Levantine viper (*Macrovipera lebetina obtusa*), including providing information on the size and number of eggs in clutches, as well as the relationship between the number of eggs in clutches and their size and weight. It has been experimentally proven that it is by the day of oviposition that embryos acquire the greatest resistance to temperature factors of the external environment. The embryos are most protected in eggs of a turtle with a hard shell (*Testudo graeca*), eggs are laid in the early stages of embryonic development (beginning and middle of gastrulation). However, in the Levantine viper (*Macrovipera lebetina obtusa*), the eggs are covered only with a fibrous membrane and the laying of eggs occurs at later stages of embryonic development (the stage of laying the tongue). It was also identified that in both species the ranges of morphological variability of embryos at the time of oviposition, in populations living at different heights, are the same. The tactical variability in the reproduction of oviparous reptiles is thought to be a result of their evolutionary adaptation to land reproduction. The incubation of eggs in the external environment lasted 80-85 days in the tortoise and 50-55 days in the viper. This is because in turtles, eggs are laid earlier, and in the Levantine viper at later stages of embryo development. In the Levantine viper, a significant part of the development of the embryos (30-35 days) occurs while the eggs are in the oviducts of the female.

Keywords: Adaptive meaning, common tortoise, embryos, Levantine viper, oviposition

Introduction

The diversity in the strategy and tactics of reproduction of the first terrestrial vertebrates - reptiles is the result of their evolutionary adaptations to the terrestrial way of life. In the course of the evolution of reproductive activity, various adaptive forms arose in reptiles, which contributed to their wider distribution on land. The general achievement of reptiles for the development of land and the acquisition of the ability to reproduce outside the aquatic environment was the formation of a special type of egg in them. This egg had all the necessary structural and organizational elements and material resources for the autonomous development of the embryo right up to the hatching of a new generation. The emergence of a new type of egg became possible due to the biological progress of reptiles along the path of aromorphosis (morpho-physiological changes), which allows them to establish new relationships with the environment (Shmalgauzen, 1969). The fate of the eggs, inside which the development of the embryo takes place, in the process of evolution for different representatives of reptiles has developed in different ways (Zusman, 1973). Taking into account the peculiarities of the structure of eggs and the levels of protection of the developing embryo, as well as the environmental conditions, evolution predetermined different reproduction strategies for different representatives of reptiles, and they became oviparous, ovoviviparous, or viviparous. For each strategy, different types of reptiles show different tactical options (Tuniev, 2008). Among the representatives of oviparous reptiles, turtles (*Testudines*) lay eggs at the early stages of embryogenesis, and snakes (Ophidia) lay their eggs for a long time and lay them at later stages of embryonic morphogenesis (Korneva, 1976; Shine, R., 2006; Gao, Jian-Fang, 2010; Anthony R. Rafferty et al., 2012; Almeida-Santos et al., 2017). The breeding tactics of oviparous species of reptiles consist of the strict confinement of oviposition to certain stages of embryo development (Korneva, 1976; Iskenderov, 1978; Najafov et al., 1994: Blackburn Daniel G., 2003). Interests are the adaptive value of the reproductive tactics of oviparous species and its variability in populations living at different altitudes. This article is devoted to the experimental study of the adaptive value of the confinement of oviposition to certain stages of embryo development and the level of morphological variability of embryos at the time of oviposition in different populations of turtle and viper.

Material and methods

The research was carried out in 2016-2017 in the experimental base (the serpentaria) of the Institute of Zoology of the National Academy of Sciences of Azerbaijan. Freshly laid eggs and embryos of the common tortoise (*Testudo graeca ibera* Pallas, 1814) and the Levantine viper (*Macrovipera lebetina obtusa* Dwigubsky, 1832) served as materials for the study. To collect materials in 2016 - 2017 during the breeding season (May-June) and until the start of oviposition. In total, 35 specimens of the turtle (♀♂) and 42 specimens (♀♂) of the viper were caught on the territory of the Akhsu district. Of these, 19 specimens of the turtle and 27 specimens of viper were caught in the

lowland (height 50-60 m), 14 specimens of turtles and 15 specimens of the viper were caught in mountainous areas (height 550-600 m). Individuals were identified by morphological features according to the method of Darevsky et al. (1989).

Turtles and vipers were kept in separate open enclosures with an area of 25–30 m^2 each and under conditions close to natural (Fig. 1). In the enclosure where the turtles were kept, a layer of soft soil 40-50 cm thick was laid for laying eggs. The animals were constantly visually monitored and the eggs laid were taken immediately. Were collected a total of 55 turtle eggs and 98 viper eggs. The eggs were measured (length and width) and weighed. Part of the eggs (1-2 pieces from each clutch) were opened to clarify the stage of development of the embryo by the time of laying, part was used for the experiment to study the thermal stability of the embryos, the rest of the eggs were incubated until hatching. Artificial incubation of eggs was carried out according to the method of T.M. Iskenderova (1978).

Figure 1. Open enclosure for keeping reptiles.1- underground shelter; 2- above-ground shelter.

Results and discussion

Most species of the herpetofauna of Azerbaijan, as inhabitants of a hot climate, lay their eggs (Alekberov, 1978). The laying time of eggs in the common tortoise and the Levantine viper varied depending on the relief, climate, and habitat. Since, in the common tortoise in the lowlands, at an altitude of 50-60 m, egg-laying began in early May (05 May 2016) and continued until mid-June. And the turtles caught in the high mountain steppes (height 600 m) started laying eggs 10-15 days later, in mid-May (May 14-16, 2016), and continued until the end of June.

Laying of eggs occurred mainly in the morning before 11 and in the evening until 19 hours. The process of oviposition, including digging a hole, lasted 1–2.5 hours (Fig. 2, a, b). The depth of the

hole did not exceed 12-15 cm and depends on the size of the female. The number of eggs in clutches varied from 2 to 10 (Table 1). The size and weight of eggs in the clutch range: length 27.5–46.5 mm (average 37,7 ± 4,2), width 24.8–42.5 mm (average 41.7±1.23), weight 17.8–26.5 g) (on average 21.9±1.9). There is an inverse correlation between the number of eggs in clutches and their sizes, as well as mass (r = - 0.864; - 0.761 and - 0.781) and this correlation is significant (t = 3.5; 2.4; 2.8 and $P < 0.05$).

Figure 2. Mediterranean turtle (*Testudo graeca ibera* Pallas, 1814): left- digs a hole, middle- lays eggs, and right-embryo at the time of oviposition at the stage of formation of the chordo-mesodermal canal. Azerbaijan, Akhsu region.

Several eggs in clutches, the less their weight and size Some of the collected eggs were artificially incubated until the young hatched. Sand with a relative humidity of 70-75% was chosen as the substrate. The eggs were placed inside the substrate, which was moistened from time to time. The temperature was maintained at 29-31°C during the day and 26-27°C at night. Incubation lasted 80-85 days. At the end of the study, animals and young were released into the wild in the places of their capture.

Table 1. Number, mass, and size of eggs in clutches of Mediterranean tortoise (*Testudo graeca ibera*) and Levantine viper (*Macrovipera lebetina obtus*a).

SPECIES	NUMBER OF EGGS IN CLUTCHES, N	EGG MASS, G, M±M	EGG LENGTH, MM, M±M	EGG WIDTH, MM, M±M	R, T, P
Testudo graeca	5	20,7 ± 0,6	37,0 ± 1,0	35,7 ± 1,1	
	5	21,9 ± 1,7	35,6 ± 0,98	29,2 ± 0,95	- 0.864;
	5	20,8 ± 0,9	36,0 ± 0,98	33,4 ± 0,75	- 0.761
	6	24,3 ± 0,6	34,8 ± 1,2	34,5 ± 1,4	- 0.781
	8	18,6 ± 0,8	29,9 ± 0,91	26,9 ± 1,04	
	10	17,8 ± 0,8	27,5 ± 1,4	24,8 ± 1,0	3.5
	2	26,5 ± 1,7	46,5 ± 1,3	42,5 ± 1,35	2.4
	3	24,7 ± 0,8	43,6 ± 1,34	40,3 ± 1,83	2.8
	4	21,3 ± 0,7	40,5 ± 1,05	37,8 ± 1,75	
	4	20,5 ± 0,4	38,9 ± 0,82	35,5 ± 1,05	P < 0.05
	3	23,4 ± 0,9	44,2 ± 1,2	41,7 ± 1,23	
	n = 11	**21,9 ± 1,9**	**37,7 ± 4,2**	**34,8 ± 4,2**	
	8	17,2 ± 0,6	36,4± 0,8	25,4 ± 0,8	
	8	18,3 ± 1,7	38,6 ± 1,8	23,4 ± 1,3	- 0.913
	11	16,8 ± 1,2	38,4 ± 2,8	24,6 ± 0,9	- 0.983

Macrovipera lebetina	12 14 15 6 6 5 4 4 5 **n = 12**	17,4 ± 0,7 16,5 ± 0,9 16,5 ± 1,5 19,5 ± 0,9 20,8 ± 2,6 23,1 ± 1,8 25,6 ± 2,1 26,2 ± 2,2 16,5 ± 1,5 **20.7 ± 2.8**	37.5 ± 0,9 36.8 ± 1.9 36,3 ± 3,6 47,9 ± 1,2 48,6 ± 3,2 49,1 ± 2,0 50,7 ± 2,1 53,4 ± 2,8 36,3 ± 3,6 **43.7 ± 5.8**	23,9 ± 0,5 22,8 ± 1,1 21,9 ± 2,8 29,4 ± 1,3 28,5 ± 1,6 31,6 ± 1,3 32,8 ± 1,9 33,1 ± 2,1 21,9 ± 2,8 **27,3 ± 3.3**	- 0.976 4,0 P < 0,001

At the Levantine viper, oviposition begins at the end of May June and continues until the end of July (Alekberov, 1978). Among the vipers caught in the lowlands (height 50-60 m), egg-laying was noted by us in mid-June (June 10, 2017) and continued until June 22. However, among the vipers caught in the foothills (altitude 550-600 m), egg-laying began late at the end of June and continued until the end of July. Egg-laying took place in the evening or at night, it was one-time or protracted, portioned. The oviposition process lasted 0,5–2.0 hours. The number of eggs in clutches varied from 4 to 15 (Table 1). Average sizes and weight of eggs in clutches range: length 36.3-53.4 mm (average 43.7 ± 5.8), width 21.9-33.1 mm (average 27.3±3.3), mass 15.5-26.2 g (average 20.7±2.8) (Table 1). In vipers, there is also an inverse correlation between the number of eggs in clutches and their size and mass (r = - 0.913; -0.983; -0.976) and this correlation is highly reliable (t = 4,0; P < 0,001).

As a substrate for the incubation of viper eggs was used sterilized wood shavings or sawdust were with a relative humidity of 75-80%. The eggs were placed in the substrate and placed in incubator. The substrate was moistened from time to time, the temperature was maintained at 29-31°C during the day and 26-27°C at night. Incubation lasted 50-55 days. At the end of the study, snakes and hatchlings were released into the wild in the places of their capture.

Our studies have shown that the breeding tactics of the common tortoise (*Testudo graeca ibera*) and the Levantine viper *Macrovipera lebetina obtusa*) consists of confining the oviposition to certain stages of embryonic development. The common turtle lays eggs in the early stages of embryonic development, namely in the period from the beginning to the middle of gastrulation (Iskenderov, 1978). Turtle embryos reach these stages at 3 days of age (Fig. 2, c). Even the over the maturation of females ready for laying for some unknown reason for some time (1-5 days) does not cause movement in the development of the embryos.

In the Levantine viper, oviposition is confined to the later stages of morphogenesis - the pre-fetal stages (Iskenderov, 1978). It was found that by the time of oviposition, the embryos of the vipers are at the stage of laying the tongue and these stages are reached by the embryos on the 33-35 day of finding the eggs in the oviducts of the female (Fig. 3, c). An attempt was made to experimentally confirm the adaptive significance of the confinement of oviposition to strictly defined stages of

embryo development in turtles and the Levantine viper. The experiment showed that early turtle embryos in eggs prematurely extracted from the oviduct do not tolerate hypo- (from +15 to +17^{0}C) and hyperthermia (from +36 to +38^{0}C), since these embryos die during further egg incubation. In addition, the embryos of natural and mature clutches successfully withstood temperature effects and developed normally during subsequent incubation.

Thus, it has been experimentally proved that early embryos of turtles, the eggs of which are equipped with a hard shell and a thin albuminous membrane, achieve certain lability of embryos to temperature effects by the time of oviposition. This is the adaptive significance of the confinement of oviposition to the early stages of embryo development in turtles.

Figure 3. Levantine viper (*Macrovipera lebetina obtusa* Dwigubsky, 1832) (left), its eggs (middle), and the Embry at the time of oviposition at the stage of tongue formation (right). Azerbaijan, Akhsu region.

A similar experiment was carried out with viper, and the temperature resistance of early embryos before oviposition was determined. 2-hour maintenance of pregnant viper females at hypo- (from +15 to + 17^{0}C) and hyperthermia (from +36 to + 38^{0}C) before oviposition led to the death of their pre-fetus during further incubation. However, embryos in natural clutches successfully withstood hypo- and hyperthermia; during the subsequent incubation of eggs, they developed normally. This indicates that the maximum temperature resistance of viper embryos is achieved precisely at the time of oviposition and at later stages of development (the stage of laying the tongue). The achievement of the required temperature stability by embryos at the late stages of morphogenesis and the confinement of oviposition to these stages are associated with the structural features of snake eggs. Due to the soft outer shell and the absence of a protein shell in the eggs of snakes, embryos are poorly protected from the harmful effects of the environment. This circumstance forces the females to bear eggs until the later and heat-resistant stages of embryonic morphogenesis. This is the adaptive feature of the breeding tactics of oviparous snakes.

We studied morphological variability in the development of embryos in freshly laid eggs in turtle and viper inhabiting different landscape zones (lowland, foothills, and mountains). It turned out that in populations of different heights, eggs are laid in the same range of morphological variability of embryo development: in the turtle, the beginning and middle of gastrulation, and in the viper, the

stage of tongue formation. It is these reproductive tactics that promote the reproduction of populations at different altitudes.

Conclusion

Thus, the adaptive significance of the breeding tactics of the common tortoise (*Testudo graeca*) and Levantine viper (*Macrovipera lebetina* obtusa) has been studied experimentally. It consists of the confinement of oviposition to the stages of development of embryos that achieve the highest resistance to temperature influences of the external environment. In a turtle, eggs, which have a hard shell and are reliably protected from external influences, are deposited in the early stages of development (beginning and middle of gastrulation). However, in the Levantine viper, the eggs are covered with a fibrous membrane and the embryos are less protected from external temperature influences and are deposited at the later stages of morphogenesis (tongue laying). The range of intraspecific morphological variability in the development of embryos by the time of oviposition in the common tortoise and the Levantine viper allows them to preserve this breeding tactic in all populations living at different altitudes. It was also established that in both species the ranges of morphological variability of embryos at the time of oviposition, in populations living at different heights it does not change. Thus, tactical variability in the reproduction of oviparous reptiles is the result of the structural and functional characteristics of eggs that have arisen in the process of evolution.

Tactical variability in the reproductive life of the common tortoise and of the Levantine viper also manifests itself in the timing of the natural incubation of their eggs. In the Levantine viper, incubation is completed 30-35 days earlier than in the tortoise. This is because viper eggs are laid at later stages of embryonic morphogenesis (the stage of tongue formation). Therefore, in the Levantine viper, a significant part of the development of the embryos (30-35 days) occurs while the eggs are in the oviducts of the female.

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