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Reproductive activity of *Lacerta agilis* and *Zootoca vivipara* (Reptilia: Sauria: Lacertidae) in western Siberia

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Abstract. From 2001 to 2005 the main reproductive characteristics of 180 specimens of *Lacerta agilis* and 282 specimens of *Zootoca vivipara* were studied using common zoological, histological, cytological and immunohystochemical methods. The maximum duration of the activity period of both species is about 4.5 months per year. Emergency from hibernation depends on the course of spring, biotope, age and sex of the lizards. Males of both species emerge from hibernation 10-15 days earlier than females. The emergency from hibernation of the first breeding males and females of *L. agilis* and *Z. vivipara* occur simultaneous with the older individuals of the same sex. However, spermatogenesis, vitellogenesis, laying of eggs in *L. agilis* or birth of young in *Z. vivipara* occur in 1-3 weeks later than at older specimens. In both species, the reproductively active part of the populations mainly included specimens after their third time of overwintering, whereas only a small portion reproduced after a second time of hibernation. Spermatogenesis in both species could be observed from beginning of May until mid of June. Testicles of both species reach maximal sizes at the moment of the appearance of the spermatozoa (beginning of May) and have minimum size in mid of summer. In both species, all mature females of a population took part in reproduction. Fecundity of females is correlated with snout-vent length. The phenomenon of long-term spermatozoid deposition in female oviducts is confirmed.

Introduction

Lacerta agilis and *Zootoca vivipara* are among the most widespread species of lizards in the northern parts of Eurasia. The southeastern part of West Siberia is at the northern limits of the range of *L. agilis* and at the centre of the *Z. vivipara* range. The reproductive biology of both species has been studied comprehensively in European regions of their areals (e.g., Jablokov, 1976; Bischoff, 1984; Saint-Girons, 1984; Amat et al., 2000; Roig et al., 2000). In contrast, there are only few data about features of sexual cycles, linear sizes of sexually mature species, their fecundity and age of sexual maturity in Asian parts of the species' range (e.g., Popoudina, 1976; Kuranova, 1983; Orlova et al., 2005).

Materials and methods

During the years 2001-05 research was conducted in the southern part of the taiga zone (53°30´ N, 84°50´ E) where both species occur. 180 specimens of *L. agilis* (84 females, 96 males) and 282 specimens of *Z. vivipara* (137 females, 145 males) were studied. The age of lizards was determined on behalf of longitudinal (thin) sections of tubular bones in polarized light of a microscope (see Kornilova et al., 1996). Sexual maturity was determined by the size of lizard (snout-vent length = SVL), its colour, and the state of gonads. Age and seasonal dynamics of testicles is described by index L. tes/SVL and D,tes./ SVL (L.tes. = length of testicle, D.tes. = diameter of testicle). Using a standard histological technique 205 preparations of reproductive system of *L. agilis* and *Z. vivipara* were made. The 3-chromatic method of preparations of Mallori was used for dyeing.

The fecundity was defined based on number of eggs present in oviducts, an egg clutch or size of spawn. Spermatozoids in urogenital organs of females were revealed by means of marked antibodies (Ackerman et al., 1988; Kremer and Jagers, 1992). Mathematical processing was carried out by means of the software package STATISTICA 6.0 and Microsoft Excel 2000. Degree of correlation of parameters is defined by Spirman's coefficient (r_s).

Results and discussion

Sexual maturity of Lacerta agilis

Males: In the study area most of the males became sexually mature after the third hibernation period and at the minimum SVL of 66.8 mm (established by histological methods). Some males already took part in reproduction after the second emergency of hibernation (SVL_{min} = 57.5 mm, 8.3%). Growth of testicles of sexually mature specimens is slowed down but not

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stopped with older age. The increase of gonad length until the end of an individual life is equal to 12% and 4% concerning the diameter (n = 66, sad-1: L.tes./L. = 0.09±0.01, D.tes./L. = 0.04±0; ad: L.tes./L. = 0.10±0 – 0.12±0, D.tes./L. = 0.05±0). The increase of SVL is accompanied by proportional increase of testicle length (r_s = 0.35, p <0.01).

Females: All sexually mature females took part in reproduction after the third emergency of hibernation (n = 11), and most of them (56.5 %) have vitellogenesis only in the spring after the third emergency of hibernation. The minimum SVL of fertilized females of this age (n = 27) is 67.2 mm.

Sexual maturity of Zootoca vivipara

Males: Sexual maturity was noted after the third emergency of hibernation (n = 98, SVL_{min} = 48.9 mm). From birth till sexual maturity (after the third emergency of hibernation) there is an intensive increase of testicles length. Already after the second emergency of hibernation the diameter of testicles increase proportionally to the SVL (n = 62, sad-1: L.tes./L. = 0.09±0, D.tes./L. = 0.04±0; ad: L.tes./L. = 0.10±0 – 0.11±0, D.tes./L. = 0.05±0).

Females: Sexual maturity of most females (n = 28, 87.5%) was noticed after the third emergency of hibernation, and in 12.5% of individuals after the second one.

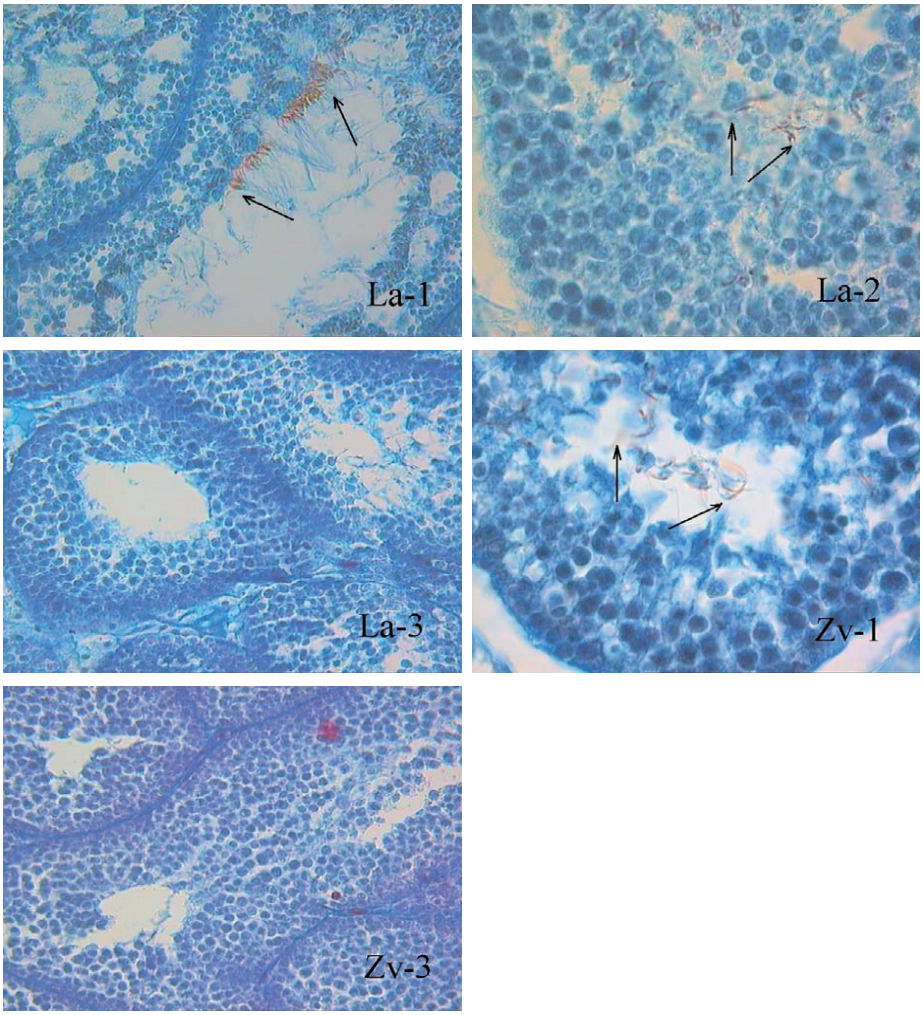

Figure 1. Testicles of *Lacerta agilis* (La) and *Zootoca vivipara* (Zv) at the beginning (the end of May: 1 mature male, 2 - male participating in reproduction for the first time) and at the end (3 - first half of August) of the activity period (magnification 40x/0.65 (La-1, La-3, Zv-3), 100x/1.25 (La-2, Zv-1)). Arrows indicate spermatozoids.

Thus, in the study area *L. agilis* got sexually mature slightly later compared to regions in the center of its areal, and simultaneously when compared to populations from the western and northwestern periphery of its range (see Jablokov, 1976; Glandt, 1988; Amat et al., 2000). In *Z. vivipara* maturity during the first 2-3 years was documented in most populations from its range (Roig et al., 2000; Orlova et al., 2005).

Phenology of reproduction

The emergency from hibernation and the dates of the main stages of reproductive cycles depend on the spring weather conditions and the biotope. The duration of the activity period of both species is about 4.5 months per year. Sexually mature males of both species are the first ones who emerge from hibernation from end of April to beginning of May. The emergency from hibernation of females of both species is 10-15 days delayed compared to males. Mass copulations takes place in the middle of May. The emergency from hibernation of the first breeding males and females of *L. agilis* and *Z. vivipara* occur simultaneous with the older individuals of the same sex. However, spermatogenesis, vitellogenesis and egg laying in *L. agilis* or birth of young in *Z. vivipara* occur within 1-3 weeks.

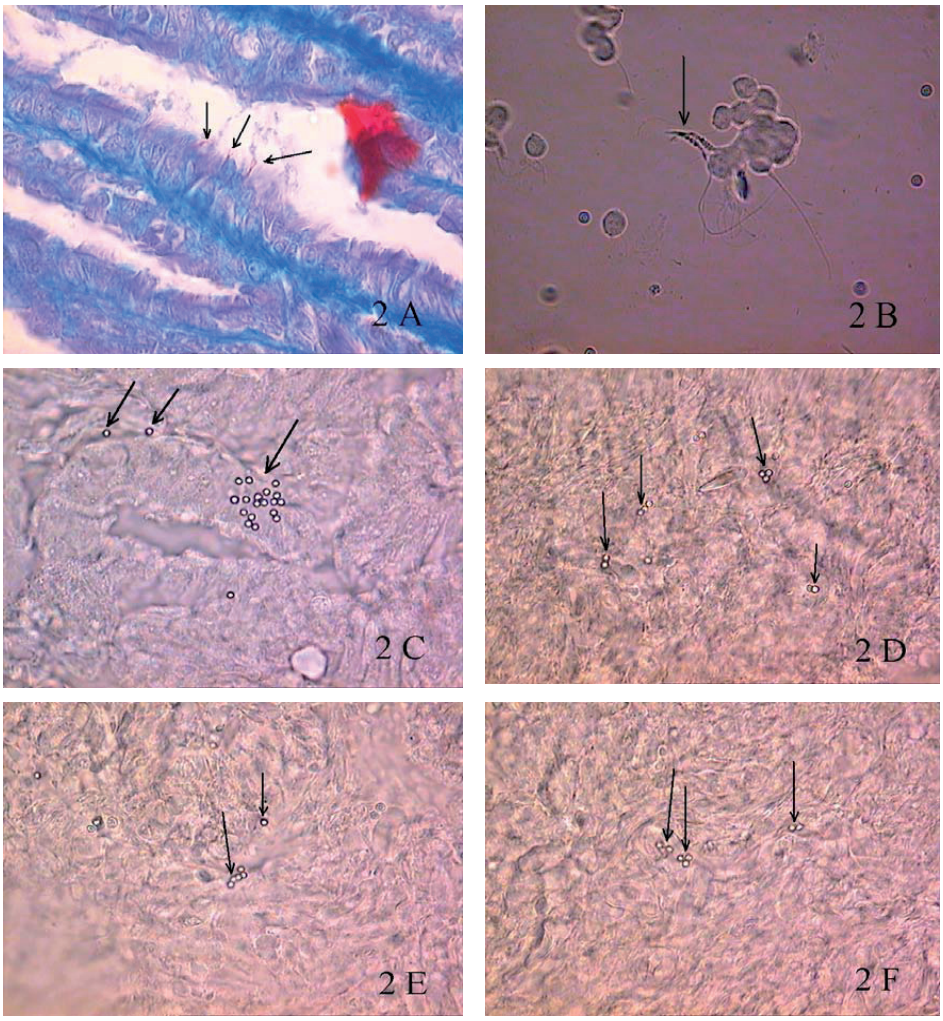

Figure 2. Depositing of spermatozoids in the genital tracts of female *Lacerta agilis* and *Zootoca vivipara*: 2A – few spermatozoids in the lower parts of the oviduct of female *L. agilis* few hours after copulation (magnification 40x/0.65); 2B - cytologic preparation of testicles of *L. agilis* with spermatozoids marked by antibodies (magnification 100x/1.25) (magnification 100x/1.25). Location of deposition of spermatozoids in female's oviducts marked with antibodies: 2C – *L. agilis*, before copulation, middle of May; 2D - *L. agilis*, after egg laying , middle of June; 2E - *Z. vivipara*, after copulation, end of May; 2F - *Z. vivipara*, middle of August (polarized light, magnification 40x/0.65).

Sexual cycles

Males: Both species have spermatogenesis of the mixed type which has a similar character allover the species' geographical range. Summer spermatogenesis and spring spermiogenesis was accompanied by copulations and ovulations at the end of spring (Saint-Girons, 1984; Amat et al., 2000). Spermiogenesis is very short and was observed only during spring time. The spermiogenesis of both species was documented from the beginning of May till the middle of June (Fig. 1). Its frequency was unstable in spring, increased from the beginning till the end of May (reaching a maximum) and gradually decreased from the beginning to the middle of June. The maximum number of spermatozoids in the testicles was detected in the second half of May. During other times of the active period spermatozoids were not found in prepared testicles (Fig. 1). The spermiogenesis of specimens taking part in reproduction for the first time is shorter and less intensive than that of older individuals (Fig. 1).

The length of testicles in both species is at a maximum when mature spermatozoids will appear (in the beginning of May) and is minimum in the middle of the summer. Reduction of testicle length was observed within 10 days after emergency from hibernation (*L. agilis*: L.tes.$_{max}$/SVL = 0.12±0.001; *Z. vivipara*: L.tes.$_{max}$/SVL = 0.12±0.008), in 10 days - diameter (*L. agilis*: D.tes.$_{max}$/SVL = 0.06±0.001; *Z. vivipara*: D.tes.$_{max}$/SVL = 0.07±0.015). Reduction was observed till the middle of June (*L. agilis*: L.tes.$_{min}$/SVL = 0.08±0.002, D.tes.$_{min}$/SVL = 0.03±0.001; *Z. vivipara*: L.tes.$_{min}$/SVL = 0.08±0, D.tes.$_{min}$/SVL = 0.04±0.001). The increase of testicle size was observed from the end of June till the beginning of August (*L. agilis*: L.tes./SVL = 0.01±0.002; D.tes./SVL = 0.04±0.005; *Z. vivipara*: L.tes./SVL = 0.11±0.003; D.tes./SVL = 0.05±0.002).

Females: In the middle of May oocytes in different stages of development were present in the ovaries of females (in *L. agilis,* oocyte diameter 0.8-7.5 mm; in *Z. vivipara*, oocyte diameter 0.6-4.7 mm). From the middle of May till the beginning of June mass copulations of lizards were observed. Efficiency of copulation is very high as all investigated mature females in both species took part in the reproduction.

The fecundity of females of both species depends on length of a body. Total number of *L. agilis* females (n = 27) contained an average number of eggs of 6.9±0.5 (3-13) (r_s=0.64, p <0.001). Of them, young females (n=11) with SVL of 71.4±0.8 (67.2-74.6) mm contained 4.9±0.3 (3-7) eggs; elder individuals (n=16) with SVL of 88.3±1.8 (75.0-104.9) mm contained 8.1±0.6 (3-13) eggs. Females of *Z. vivipara* (n=77) with SVL of 61.0±0.6 (46.2-81.0) mm contained 6.5±0.2 eggs (1-14) (r_s=0.62, p <0.001).

Deposition of spermatozoids

Investigation of the reproductive system of one female of L. agilis revealed the presence of follicles with a diameter of 4.1-8.8 mm in the ovaries as well as the presence of spermatozoids in the lower parts of its oviducts. Few hours after copulation an insignificant number of spermatozoids freely settled in gleams of the lower parts of the oviducts; most of the spermatozoids took root by means of their heads in folds of a mucous membrane and of the secretory epithelium (Fig. 2A).

Deposited spermatozoids were changed with the head being shortened and the terminal filament being absent. In the middle and upper parts of oviducts no spermatozoids were present. The modified spermatozoids were preserved in the epithelium and were not visible upon histological preparations, but were detected using marked antibodies. Immunohistochemical reactions showed that the used antibodies were connected with the head and the cervix of spermatozoids (Fig. 2B).

During investigation of females of both species during all periods of their activity the phenomenon of deposition of spermatozoids was always observed in the epithelium and in the basal membrane of the lower and the upper parts of the oviducts, whereas they were lacking in middle parts of oviducts. Spermatozoid deposition of female *L. agilis* and *Z. vivipara* were evident directly after copulation, laying of eggs or birth of young, before emergency from hibernation and in spring prior to copulations (fig. 2C, D, E, F), namely throughout the year. These observations confirm the phenomenon of earlier penetrations and spermatozoid deposition in females of *L. agilis* as described by Genin (1955).

The phenomenon of long-term storage of sperm in female oviducts is known in various groups of reptiles. Preservation of capable spermatozoids provides sperm competition while plural copulation occur, or fertilization of repeated laying and is an obligatory component of reproduction of the species having asynchronous reproductive cycles (e.g., Olsson et al., 1994; Blackburn, 1998; Gist and Congdon, 1998).

In conclusion, in the study area sexually mature individuals of *L. agilis* start being active simultaneously with peripheral populations from European regions of its range; *Z. vivipara* reaches sexually maturity at the same time as any known populations within its geographical

range. To a great extent, beginning of sexual maturity and basic reproductive characteristics are determined by climate conditions of previous years. The duration of the activity period, namely the time of reproduction of both species and its intensity depends on the biotope and climate conditions of the respective year.

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