Reproductive cycle of male wall lizard, *Podarcis vaucheri* (Reptilia: Sauria: Lacertidae), in Djurdjura, Northern Algeria

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The reproductive cycle of male wall lizard *Podarcis vaucheri* (Boulenger, 1905) living in Djurdjura Mountain, was analyzed from April until October 2014. Its seasonal mixed-type cycle was characterized by three phases: sexual activity, quiescence and recrudescence. After the emergence of lizards from hibernation, an intense sexual activity was observed in spring (April, May, and June), at which the testicular weight and seminiferous tubules diameter reached their maximal values. At this period, spermiogenesis was also maximal and the spermiation allowed the release of a large quantity of spermatozoa in both the seminiferous tubules and epididymis lumen. In July, a short sexual resting period followed, during which the testes mass and seminiferous tubules diameter decreased. Spermatogonia and some primary spermatocytes were observed against the wall of seminiferous tubules. The recrudescence period (August, September and October) was characterized by a progressive increase of the testis weight and seminiferous tubules diameter. Spermatocytogenesis began in August, and spermiogenesis occurred in September and October, before the diapause period. However, the presence of spermatozoids was not signaled in the epididymis lumen. Field observations and microscopic evidence of testis showed that both sexes reproduced synchronously during spring and early summer.

**Key words**: Algeria; Djurdjura; lizard; *Podarcis vaucheri*; reproductive cycle; spermatogenesis.

Reproduction imposes high-energy requirements, illustrated by a large diversity in terms of resource acquisition and allocation strategies (Jönsson, 1997). Seasonal breeding represents a valuable strategy in order to use this energy economically, but the reproductive timing becomes one of the most critical issues in the lizard’s biology (James & Shine, 1985). For that, the reproduction occurs when the environment is the most favorable to offspring survival and when parents can physiologically sustain reproduction with the lowest cost (Whittier & Crews, 1987; Pianka & Vitt, 2003). Timing of reproductive activity is widely variable in lizards (Manríquez-Morán et al., 2005; Carretero, 2006; Villagráñ-Santa Cruz et al., 2009; Gröbner, 2011): in temperate-zones, breeding takes place in spring and early summer, with the reproductive season being longer...
in coastal than in continental populations (Carretero & Llorente, 1995, 1997; Galán, 1997; Carretero et al., 2006a, Al-Amri et al., 2012), while in cold and high-altitude environments, mating occurs later in the season and lizards exhibit a short breeding period (Saint-Girons & Duguy, 1970; Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988; Braña et al., 1990; Olsson & Shine, 1999; Roig et al., 2000; Arribas & Galán, 2005). Further, in desert environments reproduction can be postponed as well as anticipated compared to temperate zones (Hammouche & Gernigon-Spychalowicz, 1996; Hammouche et al., 2007; Goldberg, 2013), and can continue until autumn (Goldberg, 2013). Lastly, it is in tropical areas that lizards exhibit the highest variability of reproductive patterns, ranging from continuous to seasonal reproduction (Fitch, 1970; James & Shine, 1985; Jenssen & Nunez, 1994; Pianka & Vitt, 2003; Huang, 2010; Méndez-de la Cruz et al., 2013).

Besides the environmental conditions, several studies showed reproductive cycles to be influenced also by the interaction between endogenous and exogenous factors (Licht et al., 1969; Licht, 1972; Angelini et al., 1976; Lofts, 1978; Marion, 1982; Jenssen & Nunez, 1994; Carretero, 2006). On one hand, indeed, the gonadal and hormonal activity can be considered the main endogenous mechanisms controlling reproductive cycles (Díaz et al., 1994; Ikeuchi, 2004). Being partly predeterined (Angelini et al., 1976), it is also expected that these mechanism are subject to some kind of phylogenetic constrains (Dunham & Miles, 1985; Carretero, 2006), which can limit the observed environmental and geographic variability. On the other hand, the relationships between reproductive cycles and environmental variables suggest that the endogenous mechanisms can plastically respond to climatic components, such as temperature (Licht, 1971, 1973; Aldridge, 1975; Angelini et al., 1976; Marion, 1982; Flemming, 1993; Carretero, 2006; Abu-Zinadah, 2008), precipitation (Jenssen & Nunez, 1994), photoperiod (Licht, 1971, 1973; Dunham & Miles, 1985; Flemming, 1993; Carretero, 2006), or other environmental features, like food availability (Lofts, 1978; Marion, 1982; Colli et al., 2003; Domínguez et al., 2010).

The seasonal testicular cycle in lizards was described in several species (Al-Amri et al., 2013). Specifically, for temperate regions three spermatogenic cycles were described: i) the vernal or prenuptial type, where spermatogenesis occurs during spring, immediately before or even during breeding (Licht et al., 1969; Saint-Girons, 1982; Carretero, 2006); ii) the postnuptial type, where spermatogenesis is held during summer of the previous year (Saint-Girons, 1982; Carretero, 2006); iii) an finally the mixed type, where spermatogenesis begins at the end of summer and is interrupted in winter (Licht et al., 1969; Saint-Girons, 1982; Carretero, 2006). However, Saint-Girons (1982) recognized two sub-types of this cycle. The first sub-type corresponds to the classical definition, with spermiogenesis extending until spring of the following season. While in the second sub-type, there are two periods of spermiogenesis, spermatozoa are already produced in autumn of the previous season and continues in spring after hibernation. Whatever the
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cycle, in most cases spermatogenesis is synchronized with vitellogenesis and ovulation in females (Pianka & Vitt, 2003; Carretero, 2006).

Lizards of the genus Podarcis (Wagler 1830) represent a group that has evolved and diversified in the Mediterranean Basin (Arnold et al., 2007). An exemplary case is that of the Podarcis hispanicus complex, which is among the most studied European reptiles, and is characterized by a high genetic diversity (Kaliontzopoulou et al., 2011). The inclusion of genetic sequences from North African specimens has allowed the separation of P. vaucheri from the hispanicus complex, and elevating it to the species rank (Oliverio et al., 2000; Busack et al., 2005). In addition, morphological and phylogenetic studies of Iberian and North African populations (Oliverio et al., 2000; Busack et al., 2005; Pinho et al., 2006,2007; Labres et al., 2007; Lima et al., 2009; Renoult et al., 2010; Kaliontzopoulou et al., 2011,2012) have shown the occurrence of several evolutionary lineages suggesting that Podarcis vaucheri can be considered a species complex. Furthermore, very few information is available, especially on the ecology and physiology of this species. Notably, even if some previous studies have provided data on trophic ecology (Carretero et al., 2006b; Mamou et al., 2016), thermal biology (Veríssimo & Carretero, 2009), and parasitology (Carretero et al., 2011; Damas-Moreira et al., 2014), none of them has focused on describing the reproductive cycle of this variable species.

The aim of the present work was to describe the spermatogenic cycle of male wall lizards Podarcis vaucheri based on the histological analysis of testis, in order to provide some preliminary information about its reproductive strategy.

Material and Methods

Site of study

Tala Guilef is located in the western part of the northern slope of the Djurdjura Mountain (Djurdjura National Park), Kabylie, Algeria. It is located approximately 140 km southeast of Algiers and 45 km south west of Tizi Ouzou (36°39’ N, 4°01’ E). The region is characterized by a mountain climate influenced by the Mediterranean Sea, and belongs to the humid climate scene (Hamdine et al., 1993).

The specimens were collected in a rocky environment particularly attractive for this species, characterized by the presence of stones and rocks from the Haizer massif and bushy vegetation including: Crataegus monogyna, Crataegus laciniata, Rubus ulmifolius, Rosa canina, Rosa sicula, Prunus prostrata and Berberis hispanica. In grass stratum, we essentially found: Anthemis kabilica, Artemisia absintium, Astragalus armatus, Eryngium tricuspidatum, Euphorbia luteola and Ferula communis.

Data analysis

Adult males of P. vaucheri were collected monthly (between the tenth and the twenty-fifth day of each month; three individuals/month), from April until October 2014. The snout-vent length (SVL) of each individual was measured using a digital caliper (± 0.01 mm). After dissection, the left testes were removed, weighted with a digital balance (± 0.0001 g), and fixed with buffered formalin for 24 hours, and then preserved in ethanol 70°. The tissues
were dehydrated in a graded series of ethanol, cleared in butanol prior to be infiltrated and embedded in paraffin. 5 µm sections were stained with hematoxylin-eosin, following standard histological protocols.

Spermatogenic activity was assessed qualitatively by determining the various stages of germ cells. The presence of spermatozoa and secretions in the epididymis lumen were also noted. For a quantitative characterization of testis activity, three measures were taken: the (i) number and (ii) diameter of seminiferous tubes, and (iii) epididymis epithelium thickness. These measures were repeated on three randomly selected testis sections for each individual (Table S1).

Sections of each testis were examined and photographed using a Nikon Eclipse E400 light microscope, equipped with a Nikon digital DXM1200 camera coupled with a picture analyzer Nis-Element BR 3.1(Lucia software).

For the data analysis, the mean value of each histological parameter among the three sections of each individual was considered. All parameters were Log transformed, and comparisons between the different measurements were performed with ANCOVA, using the logarithm of SVL as a covariate. Further, to investigate the influence of body size in the gonadal development, we used a Pearson correlation between SVL and testis weight.

**RESULTS**

Mean snout-vent length (SVL) of lizards was 54.73 ± 0.50 mm, ranging between 50.85 and 58.74 mm. A positive correlation was found between testicular weight and body size (SVL) (r = 0.44, P = 0.047). The weight of testis varied significantly throughout the months (ANCOVA F6,13 = 44.51, P < 0.0001), and the spermatogenesis cycle of *P. vaucheri* might be divided into three main phases (Fig. 1): i) the phase of sexual activity (April, May, June), which was characterized by the highest weight; a short period of regression and a sexual quiescence (July), during which the testis weight decreased; a recrudescence phase (August, September, October), where the testicular weight started to increase (August), reaching its maximum in October, just before the period of hibernation (November – March).

The average number of seminiferous tubules by section oscillated between 45 and 55 (Fig. 2a), and did not vary along the season (F6,13 = 0.6, P = 0.726). On the contrary, the mean diameter of the seminiferous tubules showed a clear monthly variation (F6,13 = 9.18, P = 0.005; Fig. 2b), peaking during the breeding season (April -June). The sexual resting period was characterized by a maximal regression of the seminiferous tubules diameter with an
absence of lumen. During the period of recrudescence this parameter increased progressively again, with the lumen remaining absent in August. The evolution of the diameter of seminiferous tubules corresponded to the resumption of testicular activity interrupted in November by the temperature decrease, and followed by the beginning of hibernation.

The thickness of the epididymis epithelium showed a significant monthly variations (F$_{6,13} = 32.23$, P < 0.001; Fig. 3). During the phase of sexual activity this parameter was at maximum, while a sudden reduction of the height was observed in July, persisting until October.

The examination of histological sections revealed some variations of the different categories of germinal cells during the reproductive cycle (Fig. 4). After the emergence of lizards in spring (sexual activity), the spermiogenesis was maximal and persisted until June, so, all sperm cells categories were observed (Fig. 4a), with a decrease of spermatocytes number. The spermiation allowed the release of a large quantity of spermatozoa in the lumen of the seminiferous tubules and epididymis. Moreover, the morpho-functional characteristics of the epididymis have changed; the epithelial cells became secreting and take a prismatic shape (Fig. 4b).

In July, at sexual rest, the seminiferous tubules contained spermatogonia and some primary spermatocytes (Fig. 4c). In August, at the beginning of the recrudescence period, the seminiferous tubules showed a lot of primary and secondary spermatocytes, and some young spermatids (Fig. 5a). In September and October spermiogenesis occurred, and a large number of spermatid and spermatozoa were found in the seminiferous tubules.

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**Figure 2:** Monthly variation of the (a) mean number and (b) diameter of the seminiferous tubules in *Podarcis vaucheri.*

**Figure 3:** Monthly variation of the epididymis epithelium height in *Podarcis vaucheri.*
In spite of an active spermiogenesis in autumn accompanied with the presence of mature spermatozooids in the lumen of the seminiferous tubules, the epididymis lumen remained empty (Fig. 5d).

**Discussion**

The spermatogenic cycle of *Podarcis vaucheri* is of the “mixed-type”, in agreement with what is the dominant pattern in temperate lizards (Licht *et al.*, 1969; Saint-Girons, 1982; Carretero, 2006), especially those belonging to the genera *Lacerta* s.l. and *Podarcis* (Saint-Girons & Duguy, 1970; Angelini *et al.*, 1979; Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988; Braña *et al.*, 1990; Castilla & Bauwens, 1990; Galán, 1996; Amat *et al.*, 2000; Roig *et al.*, 2000; Carretero *et al.*, 2006a). Males of *P. vaucheri* exhibited an intense sexual activity in April after the hibernation phase, followed by an interruption of the spermatogenesis in June.

Figure 4: Testis and epididymis histological sections during the sexual activity phase (April, May, June) and sexual rest phase (July) of the reproductive cycle of *Podarcis vaucheri*. (a) Seminiferous tubules during the sexual activity phase containing all cell categories: spermatogonia (Spg), primary spermatocytes (SP), secondary spermatocytes (SS), spermatids (Spt) and spermatozoa (Spz). (b) Observation of spermatozoa in the epididymis lumen during the sexual activity phase. EL: Epithelium layer. (c) Seminiferous tubules during the sexual rest phase containing only spermatogonia (Spg) and some primary spermatocytes (SP). (d) Empty epididymis lumen during the sexual rest phase.
Then, a short sexual resting phase was observed during the hot period of the year, specifically in July and at the beginning of August. This period was characterized by the maximal decrease of the morphological characters, with a disappearance of the lumen in the seminiferous tubes, and only the presence of spermatogonia. Testicular recrudescence started early in late summer (in August) and some young spermatides were already observable. Testis weight increased progressively until the onset of hibernation; and that is accompanied with a development of the seminiferous tubules corresponding to the spermatogenic activity. These observations are in agreement with those of mountains populations, such as *Phoenicolacerta laevis* (Mahrouka-Sannine Mount in Lebanon; Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988), *P. muralis* (Orédon Biologic Station of High Pyrenees in France; Saint-Girons & Duguy, 1970), and *Iberolacerta monticola* (high and low

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**Figure 5:** Testis and epididymis histological sections during the early recrudescence phase (August) and autumnal spermiogenesis (September and October) of the reproductive cycle of *Podarcis vaucheri*. (a) Seminiferous tubules at the beginning of spermatocytogenesis during the early recrudescence phase. (b) Empty epididymis lumen during the early recrudescence phase. (c) All cell categories were observed in the seminiferous tubules during the autumnal spermiogenesis. (d) Epididymis lumen remains empty during the autumnal spermiogenesis. For abbreviations, see Figure 4.
altitudes in Asturias in northern Spain; Braña et al., 1990). However, a very early stop of the spermatogenesis was reported at the end of May in Phoenicolacerta laevis (Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988), which is not the case in P. vaucheri.

In addition to the obvious spring testicular activity, another active spermiogenesis was also found in September and October. Finally, this de- 

sision was also found in September and October, another active spermiogenesis, corresponding to the second sub-type of the mixed cycle (Saint- 

Girons, 1982). Similar results were already observed in several Mediterranean lacertids such as Podarcis muralis (Saint- 

Girons & Dupuy, 1970), Podarcis siculus campestris (Angelini et al., 1979), Zootoca vivipara (Roig et al., 2000), Podarcis bocagei (Carretero et al., 2006a), Iberolacerta monticola cantabrica (Braña et al., 1990), Phoenicolacerta kulzeri (Rizk & Nassar, 2015) and Phoenicolacerta laevis (Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988).

This seasonal spermiogenesis has been interpreted as an abortive spermatogenesis (Angelini et al., 1979), or as a potential second reproductive season (Carretero, 2006).

In addition, no follow-up was possible during winter because of the local climate characterizing the Tala Guilef sector. Snowing time remained from November until March in this region (Hamdine et al., 1993), reducing the period of lizards’ activity to seven months, and thus influencing the reproductive cycle. Wall lizard Podarcis vaucheri from Djurdjura did not exhibit any sperm storage in the epididymis during hibernation. Even though spermatozoa were already present in autumn, these re- 

mained in the testes. Hence, males can be regarded as unfertile during this period (Angelini et al., 1979; Roig et al., 2000; Carretero et al., 2006a; Rizk & Nassar, 2015). They became fertile in spring only when spermatozoa migrated to the epididymis. Moreover, during the phase of sexual activity, a hypertrophy of the epididymis resulted in an increase of epithelium height and accumulation of secretory granules in its lumen. These various substances secreted by the epithelial cells seem to perform a significant role in the spermatozoa maturation (Ferreira et al., 2009; Al-Amri et al., 2013). However, the epididymis cycle of P. vaucheri was similar to that of Podarcis bocagei (Galán, 1996), P. muralis (Saint-Girons & Dupuy, 1970), Iberolacerta monticola (Braña et al., 1990), Acanthodactylus erythrurus (Carretero & Llorente, 1995), Psammodromus algirus (Carretero & Llorente, 1997), and seems different from that of Timon lepidus (Castilla & Bauwens, 1990) and Phoenicolacerta laevis (Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988), where the secondary sexual characters are well developed in both autumn and spring. Rizk & Nassar (2015) reported also this post-hibernation activity in Phoenicolacerta kulzeri, with the presence of spermatozoa and secretory granules in the epididymis only in spring. However, the occurrence of spermatozoa in the testes and epididymis in spring and autumn was reported in Liolaemus species from Patagonian, Argentina (Medina & Ibargüengoytía, 2010).

Both the histological analysis of testes and field observations showed the reproduction period lasting three months, from
April to June. In the field, several male-female pairs were seen in copulation positions, females with recent copulation scars were found, and those captured in June were pregnant (pers. obs.). This led to think that spermatogenesis and oogenesis are synchronous, and that mating really lead to fertilization. If so, the cycle of reproduction of *P. vaucheri* would belong to the associated type, which is common in temperate climate (Hraoui-Bloquet, 1985; Hraoui-Bloquet & Bloquet, 1988; Amat et al., 2000; Roig et al., 2000; Carretero et al., 2006a). According to the definition of Whittier & Crews (1987), in the associated reproductive pattern, male mating behavior coincides with the time of the year at which gonads are enlarged and actively producing gametes, while in the dissociated reproductive pattern, spermatogenesis occurred before mating and consequently the gametes are stored until the mating period (e.g. in epididymis or spermatozoa).

In conclusion, data presented here allowed us to shed light on some of the adaptive strategies used by the wall lizard *Podarcis vaucheri* in Djurdjura Mountain. Our results showed that timing of spermatogenesis reflect the "Mediterraneity" of the reproductive strategy of this species with an adaptive response to altitudinal conditions. An early interruption of the spermatic activity, a short sexual rest and an early resumption of spermatogenic activity represent adaptations allowing compensating both the long hibernation period and the late emergence in spring (Saint-Girons & Duguy, 1970). However, comparative studies between the different evolutionary lineages will be necessary to assess the geographical variations in their reproductive characteristics.

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**References**


Díaz, J.A.; Alonso-Gómez, A.L. & Delgado, M.J. (1994). Seasonal variation of gonadal development, sexual steroids, and lipid reserves in a population of the lizard...


Jönsson, K.I. (1997). Capital and income breeding as alternative tactics of resource use in...


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