Evidence of sperm storage in Schreiber's Fringe-fingered Lizard, *Acanthodactylus schreiberi schreiberi*, from Cyprus

(Reptilia: Lacertidae)

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Abstract. Four females of Schreiber's Fringe-fingered Lizard, *Acanthodactylus schreiberi schreiberi* Boettger, 1878, from the Troodos Mountains in Cyprus were captured after mating in the field and moved into isolated terraria in the laboratory. Three of them laid two clutches and the fourth one laid four clutches without any further mating with males. The last oviposition was observed 90 days after capture in the field. This indicates that females of this species are capable of storing functional sperms for at least a period of three months. This ability may have played an important role in the distribution of the lizard into the inner island and the Troodos Mountains where it can be found in sparse populations.

Key words. Captive observation, functional sperm storage, reproduction, clutch characteristics, multiple clutches.

Introduction

The ability of females to store sperm in their reproductive tract has been described in past research for several animals including reptiles (Calsbeek et al. 2007, Cuellar 1966a, Fox 1963, Olsson et al. 2007, Ortega-León et al. 2009, Saint Girons 1962, Sever & Hamlett 2002, Villaverde & Zucker 1998), amphibians (Sever 2002, Sever et al. 1999, 2001, Steinfartz et al. 2006), fish (Potter & Kramer 2000, Storrie et al. 2008, Vila et al. 2007), birds (Birkhead 1998, Parker et al. 1989) and mammals (Rasweiler Iv 1987, Rodger & Bedford 1982, Selwood & McCallum 1987). Birkhead & Moller (1993) reviewed the available (till then) information regarding sperm storage, while Holt & Lloyd (2010) assessed several relative essays in an effort to understand the way sperm storage works.

The ways to detect sperm storage are (a) observation of the production of fertile clutches by females after isolation from males, (b) noted asynchrony between copulation and ovulation, and (c) histological observation of sperm within the oviduct (see Murphy-Walker & Haley 1996). Relative observations on sperm storage in the family Lacertidae have been made on females of *Takydromus stejnegeri* from Taiwan (Chun-Fu et al. 2004) that were found to have the ability to store sperm for a period of 41 days and on females of the related species *Acanthodactylus scutellatus* (Bou-Resli et al. 1981) that with the help of sperm storage crypts were able to keep fertile sperm for a period of four months.

Usually sperm stores are kept inside females for a period of a few days or for some months (Birkhead & Moller 1993), but in some cases this period can be exceptionally extended to

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many years (PEARSE & AVISE 2001). Although no genetic evidence was used to eliminate the possibility of facultative parthenogenesis (LENK et al. 2005), an extreme case of sperm storage in reptiles is that of the Javan Ward Snake, *Acrochordus javanicus*, that can store sperm for a period of 7 years (MAGNUSSON 1979).

Sperm storage capability was found in the genus *Acanthodactylus* by BOU-RESLI et al. (1981) who located and examined sperm storage crypts in the oviduct infundibulum of the female lizard *A. scutellatus*. Acknowledging this fact and during the study of the reproductive strategy of *Acanthodactylus schreiberi schreiberi* Boettger, 1878 in the Troodos Mountains, four mated females of the species were placed in isolation to examine the possibility of laying multiple clutches without further copulation.

Material and methods

The lizards studied were part of a small population found in the Troodos mountain range (altitude 1000 m). For capturing individuals, the "fishing" method (WILLIAM 1996) was used along with baited pitfall traps. After capture, the animals were permanently marked with the toe clipping method (FERNER 2007). Their Snout-Vent Length (SVL) was measured with a plastic ruler to the nearest 1 mm while mass was measured with a mechanical pen scale to the nearest 0.5 g.

The female reproductive state was evaluated in the field by ventral palpation for the detection of enlarged ovarian follicles or oviductal eggs. The presence of mating scars on the female ventral surface was also used (CASTILLA & BAUWENS 2000, GALÁN 1997). Gravid females were transferred to the laboratory and placed in plastic terraria measuring 35 x 23 x 22 cm. Each female was housed in an individual terrarium isolated from other males or females, for the whole reproductive period. Individuals were collected from the field during the first week of June 2010 (5-7 June) and remained in captivity for 2-5 months. They were released into the field at least one month after their last oviposition in order to be certain of the absence of further pregnancy (females that laid their second clutch in mid July were released in mid August, while the one that laid a fourth clutch in mid-October was released in mid-November).

Simulation of the environmental conditions was accomplished with medium heat insulation and the creation of a natural photoperiod. Incandescent lamps were used accordingly to adjust the timetable. In each terrarium a thin layer of sand (2-4 cm) was placed, along with a small pot of water and cardboard leaves for shelter formation. The water was changed every day and the humidity was controlled with water spraying. A moisture gradient was created by watering one side of the terraria. Each lizard was fed with Tenebrionidae larvae *ad libitum*. All terraria were examined twice a day for newly-laid eggs. The length and width of each fresh egg were recorded with a calliper to the nearest 0.1 mm and the mass with a Kern 440-33N precision balance to the nearest 0.01 g. After the measurements, each egg was placed separately in a plastic cup with 16 g vermiculite and 16 ml of water. The cups were placed in a thermo-insulation box for egg incubation. Vermiculate substrate was renewed each week and the status/growth of the eggs was recorded. The temperature in the box slightly fluctuated from 28 to 30°C. Within 24 hours of hatching, the snout-vent length of the newborns was recorded with a calliper to the nearest 0.1 mm and their mass with a Kern 440-33N precision balance to the nearest 0.01 g.

Non-parametric Spearman's rank correlation was used to determine the relationships between the clutch order and mother snout-vent length with the characteristics of eggs and offspring as well as with the incubation period. For the identification of differences among the first and the second clutch of each female, the Mann-Whitney U test was used. All statistical analysis was done with Statistica 7.0 software of StatSoft, Inc.

Table 1. Information on the reproductive output of females A-D that were kept in isolation in the laboratory and the interval of days between the first and the last laid clutch. SVL: snout-vent length, EL: egg length, EW: egg width, EV: egg volume, EM: egg mass, IP: incubation period, Interval between clutch: the days from the oviposition of the first clutch to the oviposition of the second one, Interval from capture: the days from capture to the oviposition of the second clutch.

	Female	A	B	C	D	Mean
	SVL (mm)	76	73	72	70	72.75
First clutch	No. eggs	4	3	2	3	3
	Mean EL (mm)	1.72	1.69	1.77	1.71	1.72
	Mean EW (mm)	0.97	0.99	0.93	0.99	0.97
	Mean EV (mm^3)	6.79	6.94	6.37	6.99	6.77
	Mean EM (g)	0.97	0.98	0.76	0.88	0.90
	No. offspring	4	2	2	3	2.75
	Mean SVL (mm)	39.25	39.5	39.5	38.7	39.24
	Mean Mass (g)	1.28	1.26	1.48	1.24	1.32
	Mean IP (days)	59	60	62	62	60.75
Second Clutch	No. eggs	3	2	2	2	2.25
	Mean EL (mm)	1.73	1.61	1.71	1.79	1.71
	Mean EW (mm)	0.94	0.91	0.99	0.95	0.95
	Mean EV (mm^3)	6.41	5.6	6.99	6.69	6.42
	Mean EM (g)	0.86	0.77	0.88	0.91	0.86
	No. offspring	1	2	1	2	1.5
	Mean SVL (mm)	35	37	36	34.5	35.63
	Mean Mass (g)	1.07	1.08	1.19	1.1	1.11
	Mean IP (days)	56	54	58	53	55.25
Interval between clutch (days)		29	26	41	39	33.75
Interval from capture (days)		41	39	46	42	42

Results

Four females that were captured between 5 and 7 June 2010 as gravid in the field laid their first clutch during the next 15 days. All of them were able to lay at least one more clutch without prior copulation with a male, while one of them laid four clutches in a row. Information regarding the first two clutches, of all the females, can be seen in Table 1.

Using the Spearman's rank correlation test to estimate possible correlation between the

Fig. 1. Schreiber's Fringe-fingered Lizard (*Acanthodactylus schreiberi schreiberi*) from Cyprus.

lengths of the females with mean egg and mean juvenile parameters, no correlations were found (p>0.05 in all cases) although there is a tendency for bigger females to lay more eggs in a clutch than smaller females.

The comparison among the two clutches was made with non-parametric Mann-Whitney U test (due to the limited number of data) and revealed that mean egg volume (p=0.021; Z=3.31; U=0) and mean egg mass (p=0.043; Z=2.02; U=1) in the second clutch were lower than in the first one. In addition, mean juvenile SVL, mean juvenile mass and mean incubation period were also lower in the second clutch (p=0.021; Z=3.31; U=0) in all cases.

Female B was able to lay a third and a fourth clutch in the year. Each clutch had 2 eggs but only the one egg from the third clutch was fertile and hatched successfully. Information regarding the reproductive output of female B can be seen in Table 2. No significant correlation could be found between the clutch order and the characteristics of the eggs (Spearman R correlation: p>0.05 in all cases).

Taking into consideration the day of capture and the day of the last fertile clutch, it is estimated that three of the four females that were kept in captivity managed to store sperm for a period of 42 days (range: 39-46; SD=2.94) while the fourth one kept the sperm for 90 days and laid three clutches with fertile eggs.

Table 2. Reproductive output of female B that laid 4 clutches in the laboratory. Female B was gravid when moved to the laboratory and was kept in isolation throughout the reproductive period. EL: egg length, EW: egg width, EV: egg volume, EM: egg mass, SVL: snout-vent length, IP: incubation period. *only 2 of the 3 eggs hatched successfully but all of them were fertile.

Clutches	**1st**	**2nd**	**3rd**	**4th**
Oviposition date	20/06/10	16/07/10	05/09/10	07/10/10
No. eggs	3	2	2	2
Mean EL (mm)	1.69	1.61	1.72	1.76
Mean EW (mm)	0.99	0.91	0.95	0.93
Mean EV (mm^3)	6.94	5.6	6.5	6.35
Mean EM (g)	0.98	0.77	0.86	0.8
No. offspring	2	2	1	-
Mean SVL (mm)	39.5	37	36	-
Mean Mass (g)	1.26	1.08	1.12	-
Mean IP (days)	60	54	57	-
Fertility (%)	100*	100	50	0

Discussion

In this study, gravid females of the species *Acanthodactylus schreiberi schreiberi* were kept in isolation and were able to store viable and fertile sperm for a period of 3 months. According to the literature, the prolonged storage of sperm in the female reproductive track is supported by the presence of seminal receptacles (Cuellar 1966a, Fox 1963, Saint Girons 1962) that were also found in the related species *Acanthodactylus scutellatus* (Bou-Resli et al. 1981). When females ovulate, sperm from those receptacles fertilises the ovum. According to Fox (1963) and Cuellar (1966b), the release of spermatozoa embedded in seminal receptacles is caused by the pressure exerted on the walls of the tubules during the ovulation process. This pressure is caused by the eggs passing through the infundibulum. This explanation was also adopted by Bou-Resli et al. (1981), while Birkhead & Moller (1998) state that the presence of seminal receptacles might have evolved in order to prevent sperm from being forced out of the oviduct by the first egg of a clutch.

Nevertheless, although seminal receptacles are responsible for the prolonged presence of viable sperm in the oviduct, the decrease in fertility during subsequent clutches remains a fact. Chun-Fu et al. (2004) state that this decrease might be a result of increase in sperm senility, mortality and/or passive loss. In *Uta stansburiana* (Cuellar 1966b) and *Chamaeleo hoehnelii* (Lin 1982), fertility decreases from the second to the third clutch (from 53% to 0% in the case of *Uta stansubriana* and from 84% to 62% in the case of *Chamaeleo hoehnelii*), while in *Takydromus stejnegeri* (Chun-Fu et al. 2004) that had four clutches the fertility drastically decreased from 100% in the third clutch to 33% in the fourth one. In this

Sperm storage is highly linked with sperm competition (GIST & FISCHER 1993, SCHUETT 1992), which is widespread in the animal kingdom and can occur in reptiles as well (GIBSON & FALLS 1975, HARRY & BRISCOE 1988). According to BIRKHEAD & MOLLER (1993), the longer the time interval between copulation and fertilisation, the greater the opportunity of sperm competition and the opportunity of females to modify their original choice of partner by preferentially using the sperm from the most preferred male (in case of last male precedence). In other cases, females carrying the sperm of a male can control whether to copulate or not with a newly encountering male or even copulate with several males simply to allow the sperms to compete among themselves, on the assumption that the best males produce the most competitive sperm (BIRKHEAD et al. 1993, MADSEN et al. 1992). As a consequence, sperm competition and mating with multiple males may ensure that higher quality sperm fertilises the female's eggs (HOSKEN et al. 2003, TREGENZA & WEDELL 2002) while at the same time increases the genetic diversity of the progeny (CALSBEEK et al. 2007).

In addition to sperm competition in reptiles, the presence of viable sperm in the female reproductive tract for a long period of time ensures that fertilisation can be possible in periods long after copulation. This ability is probably a physiological necessity because of ectothermy and long reproductive cycles, while it is crucial for species that are relatively immobile or that have a low density of occurrence and as a consequence have few possibilities of encountering a member of the opposite sex (CONNER & CREWS 1980, JUN-YI 1982, PHILIPP 1979, SAINT GIRONS 1982). This can be clearly seen in the case of chameleons that, compared with other more mobile lizards, can store sperm for a considerably longer period of time (BIRKHEAD & MOLLER 1993, SAINT GIRONS 1982).

Furthermore, CONNER & CREWS (1980) reported that the risk of predation may also be reduced by sperm storage, as a consequence of the reduction of copulation frequency. According to the same researchers, sperm storage is also linked to the reproductive success of females when colonising new habitats. This conclusion was also reported from studies on the Common House Gecko, *Hemidactylus frenatus* (YAMAMOTO & OTA 2006). Based on these considerations, we believe that abilities derived from sperm storage might have helped the distribution of the lizard in a variety of unfavourable ecosystems on the island as well as the establishment of locally abundant populations (OSENEGG 1989, SCHÄTTI & SIGG 1989). As reviewed by BAIER et al. (2009), *Acanthodactylus schreiberi schreiberi* can be found in a great variety of ecosystems on the island, from sandy soil ecosystems at sea level to forest ecosystems at high elevations. In addition, the advantage derived from sperm storage during colonisation might also be linked to the debate between FRANZEN (1998) and SINDACO et al. (2000), as reported in BAIER et al. (2009), regarding the origin of the *Acanthodactylus schreiberi schreiberi* population in southeastern Turkey and might add support to the introduction hypothesis (the species was introduced into southeastern Turkey from the island of Cyprus).

Considering the differences between the first and the second clutches of the four females, it becomes clear that the second one has smaller and lighter eggs and offspring. Based on the knowledge that females invest a great amount of energy in producing and developing their eggs (BALLINGER 1977, HAHN & TINKLE 1965) and that the mass of the eggs is linked to the energy invested into them (BAUWENS 1999, VAN DAMME et al. 1992), it is evident that less energy was invested in the second clutch compared to the first one. As regards the characteristics of the offspring, lower length and mass were expected since the eggs and offspring hatched from them are highly correlated amongst each other (BAUWENS 1999, FORBES & WIEBE 2010, SINERVO 1990), while the close relation between initial egg mass and the incubation period (DEEMING et al. 2006, SINERVO 1990) seems to be responsible for the shorter incubation period of the second clutch in comparison to the first one.

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