A Production of Four Successive Clutches of Eggs by a Female Grass Lizard (*Takydromus stejnegeri* van Denburgh) in Captivity

圈養中之蓬萊草蜥（*Takydromus stejnegeri*）連續四窩蛋的生產現象

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Abstract

A female grass lizard *Takydromus stejnegeri* van Denburgh was captured after mating in the field on 24 April 2003. It was immediately brought back to the laboratory and kept under observation. It produced four clutches of eggs from10 May to 22 June. The average time interval of the successive clutches was 14.75 days, and the average clutch size was 2.75 eggs. The production of the successive clutches without mating in captivity may be due to the sperm storage in the female reproductive tract. The fertilization ratio and hatching ratio were 100% for the first three clutches, but only 33.3% and none, respectively, for the fourth clutch. The clutch order was significantly positively correlated with egg width and also with egg weight. *T. stejnegeri* was an intermediate egg retainer, whose gestation (egg retention) period was at an average of 25% of the total embryonic development period.

摘　要

2003年4月24日於野外捕捉到一隻剛交配過的雌性蓬萊草蜥，隨即帶回實驗室飼養及觀察。被隔離的雌蜥於同年5月10日至6月22日間連續產下四窩蛋，此四窩蛋產出的間隔平均為14.75天，每窩平均2.75個蛋。此圈養後無再次交配的連續性生產現象，表示該雌蜥可能具有精子儲存的能力。前三窩的受精率及孵化率均為100%，而第四窩其值則分別降為33%及0%。此四窩蛋的產出順序與蛋的寬度及重量呈現顯著性地正相關。蓬萊草蜥所產下的蛋屬於居中型的胚胎發育，

其胚胎保存在雌蜥體中的平均時間約占總成長時間的25%。

Key words: *Takydromus stejnegeri*, clutch, sperm storage

關鍵詞：蓬萊草蜥、窩數、精子儲存

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Introduction

Lizards of the genus *Takydromus* (Lacertidae) are small reptiles commonly found in grasslands and bush areas. Females produce two to several clutches of eggs during a single breeding season (Loveridge 1945; Fukada 1965; Huang 1998). For reptiles when gonadal activities of both sexes are not synchronous, sperm storage is evolved (Pough *et al*. 1998), so that the sperms remain viable and fertile in female reproductive tracts for many months (Frisch 1963; Cuellar 1996a). However, there was no information whether the *Takydromus* lizards that produce successive clutches of eggs have evolved with sperm storage capacity.

As embryonic development of a reptilian egg is initiated immediately after fertilization, and the egg must be retained within female reproductive tract for a certain period for shell deposition, a considerable embryonic development has been preceded at the time of its deposition. For most lizards, approximately 12 to 73% of the embryonic development have been completed within the female bodies when eggs are laid (Shine 1983; DeMarco 1993).

Takydromus stejnegeri van Denburgh, 1912 is a small, slender, long-tailed lizard endemic to Taiwan. It is commonly found in grass and bush environments. It is an oviparous animal and its eggs are found from March to August (Lin and Cheng 1990). Clutch sizes have been reported to be 2 to 4 eggs (Cheng 1987; Lin and Cheng 1990). This study used a mated female of *T. stejnegeri* in captivity to determine the clutch frequency, clutch interval, clutch size, egg size, egg weight, fertilization ratio, hatching ratio, and percentage of embryonic development at the time of egg deposition.

Materials and Methods

On April 24 2003, an adult female *T. stejnegeri* was captured in the field near Shitan, Maioli, Taiwan (Lat. 24°33'N and Long. 120°55'E, elevation 350m). Its back and abdomen had fresh marks of being bitten with bloodstains, and thus, it was confirmed to have just mated. It was immediately brought back to the laboratory of Endemic Species Research Institute in Chichi, Nantou (elevation 240m), 80 km from the captured site. The lizard was under observation from 24 April to 11 July 2003, the period corresponding fairly well with the breeding season of *T. stejnegeri* (Lin and Cheng 1990). It was kept in a 1x0.5x0.5m (length x width x height) terrarium, in that bricks, debris, woods, grasses and a water dish were placed to mimic its natural environments. Two carton shelters and a 20x10 cm container filled with moist soil and fallen leaves were placed in the terrarium as its resting and egg deposition sites. Once a day the lizard was fed with mealworms (larvae of *Tenebrio molitor*) until it stopped feeding, and the number of mealworms consumed was recorded.

The lizard was daily palpated to determine the approximate time of its ovulation. When eggs were observed in the terrarium, they were removed and incubated in a 20x10 cm container at ambient temperature. The time of ovulation, the gestation period, and the time of egg deposition of the lizard were recorded. The size and frequency of the clutches, color, size and weight of the eggs, the fertilization ratio, the incubation period, and the hatching ratio were also recorded. Each of the eggs was weighed to the nearest 0.001g, and its length and width were measured to the nearest 0.05 mm. Total embryonic development period was the number of days from the time of ovulation to the time of hatching. The gestation (egg retention) period was the number of days for eggs to have retained in uterus before deposition. Egg incubation period refered to the number of days from the time of deposition to the time of hatching. The percentage of embryonic development period at the time of deposition was measured by the proportion of the gestation period divided by total embryonic development period. Spearman's rank correlation analysis (Krebs 1999) was used to determine the relationships between clutch order and each of egg length, width and weight, and between the egg length and the egg width.

Results

The female lizard used in this study was 73 mm SVL. Its weight varied from 4.9g to 7.2g during the study period. It laid eggs of a clutch usually within a day in a den site with moist soil and fallen leaves. After deposition, it left the site and moved to foraging areas. It consumed 0 to 4 mealworms a day with an average of about two mealworms. About three days before each of the egg depositions, its appetite decreased and ate only one mealworm, but after the deposition its appetite immediately increased and ate 4 mealworms in the first feeding. Apparently, its appetite fluctuated greatly during the breeding season in correspondence with gestation and egg deposition. It shed its skin once in the middle of each of the gestation periods.

The color of fertilized eggs changed from white to gray in correspondence with the embryonic development. An infertile egg usually remained creamy white for weeks after deposition, and then gradually became yellowish white later instead of gray. The fertilized eggs were 10.49±0.37 mm (ranged between 10.00 mm to 11.30 mm) in length, 6.46±0.30 mm (6.00 mm to 7.10 mm) in width, and 0.236±0.019g (0.207g to 0.302g) in weight. The fertilization ratios and hatching ratios were 100% for the first three clutches. For the fourth clutch, only one egg of the three eggs was fertilized but its embryo died on 8 July 2003. The clutch order was significantly correlated with egg width (r = 0.851, df = 7, $p < 0.01$) and egg weight (r = 0.714, df = 7, $p < 0.05$). The egg length was not significantly correlated with egg width (r = 0.055, df = 7, p > 0.05) and clutch order (r = -0.214, df = 7, p > 0.05),

After each of the egg depositions, we could palpate the ova in the lizard at the second or third days, and thus, the second day after the egg deposition was assumed to be the time of beginning ovulation and fertilization. The gestation period was 16, 11, 12, and 20 days, respectively, for the four successive clutches. The egg incubation period was 38 days for the first clutch, 41 and 42 days for the two eggs of the second clutch, and 40 days for the third clutch. The percentages of embryonic development period at the time of deposition were 25%$\pm$4% with a range between 20% and 30%.

Discussion

In this study the *T. stejnegeri* female was in captivity alone after mating in the field, and successively laid four clutches of eggs from 24 April to 22 June. The discovery of the second, third and fourth clutches of this female lizard without mating under captivity could only be explained by the fact that female *T. stejnegeri* stored sperms that were viable and fertile for at least 41 days.

Sperm storage has been suggested to make females less dependent on multiple mating and to ensure multiple fertilizations of subsequent clutches in the absence of males (Conner and Crews 1980; Gist and Jones 1987). The capacity for prolonged sperm storage in female reproductive tract is highly developed in reptiles (Birkhead and Moller 1998), and has been reported in several lizard species, such as *Tarentola m. mauretanica* (Picariello *et al*. 1989), *Calotes versicolor* (Ruth Shantha Kumari *et al*. 1990), *Heteronotia bionei* (Whittier *et al*. 1994), and *Psammophilus dorsalis* (Srinivas *et al*. 1995).

On the other hands, seminal receptacles may have evolved in the female reproductive tracts to prevent sperms from being forced out of the oviduct by the first egg of a clutch (Birkhead and Moller 1998). Although seminal receptacles increase the survival of spermatozoa, the decrease in fertility in subsequent clutches is also an inevitable result; only 53% of the eggs in second clutch were fertile, and 0% in third clutch in *Uta stansburiana* (Cuellar 1966b), and 84% to 62% for three subsequent clutches in *Chamaeleo hoehnelii* (Lin 1982). In this study the female *T. stejnegeri* in captivity had 100% fertility for the first three clutches but only 33.3% for the fourth (last) clutch. The decrease in sperm fertility during the storage period in the female tracts (seminal receptacles) might be due to increase in sperm senility, mortality and/or passive loss.

According to the time of mating and the duration of gonadal activity, reptiles exhibit three general types of reproductive cycles: associate cycle, dissociated cycle, and continuous cycle (Pough *et al*. 1998). For the associated cycle, gonadal activities of both sexes increase almost synchronously before mating, and then they regress out with their breeding seasons. There is no sperm storage by female. For the dissociated cycle, the breeding season is short, and the time for mating does not correspond well with the time of the female gonadal activity. The species with the dissociate cycle would evolve the storage of sperms (Pough *et al*. 1998). For the continuous cycle, gonadal activity of both sexes is sustained throughout most of a year in tropical habitats, so that no sperm storage is evolved.

In this study, the reproductive cycle of *T. stejnegeri* is likely to be the dissociated cycle. The breeding season of female *T. stejnegeri* is from March to August (Lin and Cheng 1990). The spermatogenesis occurs all the year around except for September and October (Cheng and Lin 1977; Lin and Cheng 1990). Although gonadal activities of both sexes regress synchronously, the spermatogenesis increases in November earlier than the timing of female gonadal activity. The earlier spermatogenesis might evolve to benefit male-male competition for mating, and each female perhaps mates only once.

In nature lizards are subjected to conflicting energy demands for reproduction, escaping predators, and foraging activities. In this study the female *T. stejnegeri* in captivity produced four clutches and 2.75 eggs per clutch, perhaps resulted from the absence of predator and the substantial availability of food. In lacertid lizards the first clutch size and egg size are determined mainly by energy reserves stored, but those in its successive clutches were determined by their subsequent energy intakes (Brana *et al*. 1991). In this study the female *T. stejnegeri* in captivity was provided with sufficient food supplies, so that it produced larger and heavier eggs for the latter clutches.

DeMarco (1993) proposed that sceloporine lizards may be divided into brief egg retainer, intermediate egg retainer, or extreme egg retainer, according to gestation (egg retention) period relative to the total embryonic development period at the averages of 12.7%, 29.8%, and 73.7%, respectively. In this study the gestation period relative to the total embryonic development period was averaged at 25%. Accordingly, *T. stejnegeri* may be considered as an intermediate egg retainer.

The results obtained in this study were based on the observation of a single female *T. stejnegeri* in captivity, and further studies are required particularly in the field to shed light on the reproductive pattern of this species.

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