# 中国石龙子雄性生殖腺的年周期变化\*

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摘 要 2001年3月至2002年2月期间,通过每月捕捉浙江丽水中国石龙子(Eumeces chinensis) 雄性成体,解 剖动物、观测性腺的形态和组织学特征,研究雄性生殖周期。睾丸重量和体积、附睾重、输精管重和曲细精管 直径有显著的季节变化。睾丸3月份最重,5-9月最轻。睾丸体积和重量的年周期变化规律一致。附睾3月份 最重,8-9月份最轻。输精管4月最重,8-10月最轻。生精活动始于9月下旬,翌年4月最为活跃。3月下旬 曲细精管直径达全年最大值,管腔中开始出现呈穗状排列的精子。从基膜到管腔,各级生精细胞依次排列。4月 份生精上皮的生精活动最为活跃,5月下旬生精活动已近停止,7-8月份曲细精管管壁仅由精原细胞(其间夹 有支持细胞)构成。根据曲细精管生精上皮的年周期变化规律,中国石龙子8月份睾丸生精活动处于第 期,9 月至次年2月份第 期,3月上、中旬为 期,3月底至4月为 期,5-6月份为 期,7月份为 期。4月下 旬附睾管腔中有大量的成熟精子,7月附睾管腔中已无精子。中国石龙子属于关联型繁殖周期 [动物学报 50 (1):103-110,2004]。

关键词 中国石龙子 雄性生殖周期 睾丸 附睾 输精管 曲细精管

### Annual variation in gonads of male Chinese skinks Eumeces chinensis

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**Abstract** From March 2001 to February 2002, we collected monthly 8 - 15 adult males of *E. chinensis* from a population in Lishui, Zhejiang, eastern China, to study the male reproductive cycle. The captured skinks were transported to our laboratory at Hangzhou Normal College, where they were measured, weighed, and dissected. Testes, epididymides, and ductus deferens were removed from each dissected skink, weighed, and fixed with Bouin's solution for 24 h. The fixed testes were then measured for length (2a) and width (2b), which were used in the equation of ellipsoid [V = (4/3)]

ab<sup>2</sup>] for calculating the volume of testis. Paraffin slices were prepared for testes, epididymides, and ductus deferens following routine procedures, so that the structural and morphological features of these components could be observed. Seasonal changes in testes, epididymides, and ductus deferens were very pronounced in E. chinensis. The largest testes were observed in March, and the smallest from May to September. Epididymides were also largest in March, but smallest from August to September. Ductus defference were largest in April, and smallest from August to October. A rapid increase in the thickness of the germinal epithelium occurred in late September, suggesting an initiation of testicular recrudescence. After the winter dormancy, the diameter of seminiferous tubules increased steadily and reached its peak in late March, when sperms had already appeared inside the lumen of seminiferous tubules. Spermatogenesis was very active in April, but ceased soon in late May. A larger number of matured sperms could be found in the lumen of ductus epididymis in late April, but none could be found in July. The wall of the seminiferous tubules consisted of almost a single layer of spermatogonia and Sertoli cells in July and August. Based on descriptions by Radder et al. (2001) for the annual cycle of testicular activity of lizards, annual testicular activities of E. chinensis can be divided into six stages, including Stage in August, Stage from September to February, Stage from early to mid-March, Stage from late March to April, Stage from May to June, and Stage in July. Our results, coupled with the data reported previously for adult females, show that male and female reproductive cycles of E. chinensis are associated, as spermatogenesis and vitellogenesis initiate almost synchronically and mating activities resulting in fertilization of eggs occur only in April and May [Acta Zoologica Sinica 50 (1): 103 - 110, 2004].

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Key words Chinese skinks, *Eumeces chinensis*, Male reproductive cycle, Testis, Epididymis, Ductus deferens, Seminiferous tubule

蜥蜴类动物的繁殖周期有连续型、分离型和关 联型三种类型 (Pough et al., 1998)。连续型繁殖 在无季节性变化的热带地区种类中较为普遍,精子 发生和交配几乎整年都在进行 (Somma and Brooks, 1976; Jenssen and Nunez, 1994); 分离型 繁殖在温带地区种类中较为常见,交配期短暂,受 精前精子贮存于雌性的生殖管道内 (Guillette and Sullivan, 1985; M éndez de la Cruz et al., 1988; van Wyk, 1995);关联型繁殖在温带或有季节性变化 的热带地区较为常见(Diaz et al., 1994; Censky, 1995; Huang, 1997),两性配子发生与交配活动密 切相关。温带地区具有关联型繁殖周期的蜥蜴,睾 丸年活动周期可以分成两个时相:(1)复苏期,一 般发生于春季,以最终产生精子为特征;(2)退化 期,一般始于夏末,以迅速减少并最终停止产生精 子为特征 (Fitch, 1970; Lofts, 1987; Castilla and Bauwens, 1990) 。

石龙子科蜥蜴广泛分布于热带和温带地区 (Greer, 1989), 中国石龙子 (Eumeces chinensis) 主要分布在中国南部(包括台湾、海南)和越南, 是华东和华南许多地区蜥蜴区系的重要组成成分 (Zhao and Adler, 1993)。有关中国石龙子的基础研 究数据较为丰富,内容包括亲体护卵行为(王培 潮, 1966; de Fraipont et al., 1996)、食性(王培 潮, 1964; 林植华、计翔, 2000)、行为表现和生理 功能的热依赖性(计翔等, 1995; Xu et al., 1999)、两性异形和雌体繁殖输出(林植华、计翔, 2000; 马小梅、计翔, 2001; 计翔等, 2002)、卵孵 化和幼体特征(计翔、章朝华,2001;计翔等, 2002) 等,这些积累使得该种动物更适合用作实验 模型。王培潮(1966)曾研究杭州产中国石龙子的 两性繁殖周期、但因缺乏部分月份数据、使得该种 繁殖活动的年周期变化规律仍不明确。本文作者主 要探讨雄性繁殖周期,内容涉及睾丸、附睾、输精 管和精子发生的年周期变化。

## 1 材料与方法

实验用中国石龙子均系性成熟雄性个体,采自浙江丽水三岩寺。采集地当年(2001年3月-2002年2月)的年平均气温、最高和最低月平均温度分别为18.1、29.6和6.2,年降水量为

1 427 mm (图 1,浙江省气象局)。每月下旬捕捉 8-15 条石龙子,带回杭州师范学院两栖爬行动物 实验室。捕获的动物在 24 h 内称重 (精度 0.1 g), 断头处死后剖取右侧睾丸、附睾和输精管并用 Mettler AB204-E 电子天平分别称重 (精度 0.1 mg),随后用 Bouin 氏固定液固定;左侧性腺和附 属性器官投入液氮中,后转置于-80 低温冰箱内 保存,用于测定雄性激素 (结果另文发表)。



## 图 1 浙江丽水 2001 年 3 月 - 2002 年 2 月的月平均气 温和降雨量 (浙江省气象局,未发表资料)

实线代表气温年变化,虚线代表降雨量年变化。

Fig. 1 Monthly mean air temperatures and rainfall in Lishui, Zhejiang, during March 2001-February 2002 (Zhejiang Provincial Bureau of Meteorology, unpublished data)

The solid line represents annual variation in air temperature, and the dash line annual variation in rainfall.

右侧性腺及附属性器官固定 24 h 后用 70 %酒 精洗去固定液,随后用 Mitutoyo 游标卡尺测量睾 丸长、短径(精度 0.1 mm)。睾丸、附睾、输精 管用乙醇浓度梯度脱水和石蜡包埋。在睾丸长径最 大处纵切 10 片(厚度 3 µm),HE 染色。Nikon 显 微镜观察每个切片,睾丸生精活动按照 Radder et al. (2001)的方法进行分期(表 1)。用 Image-Pro Plus 软件(Media Cybernetics, Inc)测量曲细精管 的直径。在附睾和输精管的最大直径处做横切、 HE 染色,镜检每月精子的贮存情况。睾丸体积用 椭球体公式计算:

$$V = 4/3 \quad ab^2$$

其中 V = 睾丸体积, a = 1/2 睾丸短径, b = 1/2 睾丸长径。

在做进一步统计检验前,所有数据用 Kolmogorov-Smironov和 F-max (Statistica 统计软件包) 分别检验其正态性和方差同质性。经检验,所有原 始数据均需 Loge 转换才能符合参数统计的条件。 用线性回归、协方分析 (ANCOVA)、方差分析 (ANOVA) 和 Tukey's 多重比较处理相应的数据。 动物体重是所有 ANCOVA 的协变量,比较矫正平 均值前,检验斜率的均一性。显著性水平设置为 = 0.05 °

#### 结 果 2

## 2.1 睾丸、附睾和输精管的季节变化 300

250

200

150

100

50

40

15

2 3 4 5 6 7 8 9

睾丸重量 (Testis mass) (mg)

以体重为协变量的单因子 ANCOVA 显示,中 国石龙子睾丸的重量 (F<sub>11,99</sub> = 62.49, P < 0.0001) 和体积 ( $F_{11,86} = 55.56$ , P < 0.0001)、 附睾(F<sub>11,99</sub> = 2.52, P < 0.007) 和输精管  $(F_{11,99} = 10.89, P < 0.0001)$ 的重量有显著的季 节变化(图 2A - D)。单因子 ANOVA 显示,中国 石龙子曲细精管直径亦有显著的季节变化  $(F_{11,153} = 27.25, P < 0.0001)$  (图 3)。

睾丸 3 月份最重, 5 - 9 月份最轻(图 2A); 睾丸体积的年周期变化规律与睾丸重量一致(图 2B)。输精管 4 月份最重, 8 - 10 月份最轻(图 2C)。附睾3月份最重,8-9月份最轻(图2D)。 曲细精管直径3月份最大,7-8月份最小(图3)。

10 11 12



10 11 12

Annual variation in testis mass (A), testis volume (B), ductus deferens mass (C) and epididymis mass (D) of Chi-Fig. 2 nese skinks Eumeces chinensis

Data are expressed as adjusted mean  $\pm SE$ , with body mass being set at 30 g. Means with different superscripts differ significantly (Tukey's test, = 0.05).



400

350

300

250

200 150

100

50

5

2 3 4 5 6 7 8 9

睾丸体积 (Testis volume) (mm<sup>3</sup>

#### 表 1 睾丸年周期活动的分期 (Radder et al., 2001)

Table 1The stages of testicular activity during the whole cal-endar year ( Radder et al. , 2001)

分期	曲细精管特征
Stage	Traits of the seminiferous tubule

#### 只有精原细胞

Presence of spermatogonia only

#### 有精原细胞、初级和次级精母细胞

Presence of spermatogonia and primary and secondary spermatocytes

#### 有精原细胞、精母细胞和一定数量的精子细胞

Presence of spermatogonia and spermatocytes, moderate number of spermatids

#### 精原细胞、精母细胞及大量的精子细胞和精子

Presence of spermatogonia and spermatocytes, a great number of spermatids and sperm

#### 管腔中有残留的精子细胞和精子

Presence of residual spermatids and sperm in the lumen

#### 曲细精管管壁由单层精原细胞和支持细胞所构成

Seminiferous tubules lined by a single layer of spermatogonia and Sertoli cells



#### 图 3 中国石龙子曲细精管直径的年周期变化

数据用平均值 ±标准误表示。上标不同的平均值差异显著 (Tukey 's 检验, = 0.05)。

Fig. 3 Annual variation in diameter of seminiferous tubules of Chinese skinks Eumeces chinensis

Data are expressed as mean  $\pm SE$ . Means with different superscripts differ significantly (Tukey 's test, = 0.05).

## 2.2 睾丸、附睾和输精管组织结构的季节变化

中国石龙子的睾丸主要由曲细精管组成。曲细 精管的生精上皮由各级生精细胞和支持细胞所组 成,外被基膜。生精活动活跃期曲细精管从基膜到 腔面有 1-10 层或更多层细胞,其中最外 1-3 层 是精原细胞,向内依次为初级精母细胞、次级精母 细胞、精子细胞和精子(图版 :1),生精上皮的 组成随季节而发生周期性变化(图版 :1-4)。

7、8月份,曲细精管排列疏松,管径全年最 小;生精上皮仅由精原细胞(其间夹有支持细胞) 构成,细胞核较大,染色较浅;间质相对较多(图 版 :1,6)。附睾管和输精管的管径小、管壁薄, 管腔中留有无定形物(图版 :8,10)。

9月份,曲细精管排列变得紧密,管径增大, 精原细胞开始增殖,下旬,生精上皮由近 10 层细 胞组成,管腔很小,除精原细胞外可见初级精母细 胞和次级精母细胞(图版 : 2)。附睾和输精管的 管壁同 7、8月份的相似,由一层立方体或矮柱状 细胞构成。

10 月份,曲细精管管径进一步增大,生精上 皮细胞的增殖使管腔变得更小。附睾管和输精管的 管壁有所增厚,其上皮细胞的基底部为 3 - 4 层染 色较深的细胞核,腔面有一层明显的纤毛。

11 月至次年 2 月份,曲细精管的管径明显地 增大,生精上皮增殖活动旺盛,出现了除精子细胞 和精子以外的各级生精细胞并占据整个腔面。附睾 管和输精管的管腔也明显增大,上皮细胞变成高柱 状。

2月底至3月中旬间,曲细精管中央开始出现 大量的精子细胞(图版 : 3),至该月底,曲细精 管的管径为全年最大,近管腔周围出现精子;各级 生精细胞从基膜到管腔呈穗状依次排列。此时,附 睾管管径和管壁高度也达全年最大值,附睾管腔中 有精子,但输精管中尚难以镜检到精子。

4月份,曲细精管管径虽有所减小,但生精活 动最为旺盛几乎达管壁基部,管腔的大部分被杆状 的精子占据,周边依次排列着精子细胞、次级精母 细胞、初级精母细胞和精原细胞(图版 : 4)。此 时,输精管和附睾管的管腔中有大量的精子(图版 :7,9)。

7,9)<sub>o</sub>

5月下旬,曲细精管管径明显变小,生精上皮 由一层细胞构成,管腔中有少量残留的精子,生精 活动已经停止(图版 : 5)。

6月份,曲细精管管腔被无定形物填充,腔内 仅有稀少的残留精子。此时,附睾中精子较少,输 精管中很少或几乎没有精子。

与此同时,在睾丸的年周期变化过程中,曲细

精管间的间质细胞的分布也发生规律性变化:当曲 细精管中央的主要细胞为精子细胞和精子时,间质 细胞位于曲细精管周围;当曲细精管生精上皮中只 有精母细胞时,间质细胞的分布基本上被限于三个 或三个以上曲细精管的交接处(图版 :1-6)。

综上所述,从睾丸曲细精管生精上皮的年周期 变化规律来看,8月份中国石龙子睾丸生精活动处 于第 期,9月至次年2月份为第 期,3月上、 中旬为 期,3月底至4月为 期,5-6月份为 期,7月份为 期(表1)。

3 讨 论

中国石龙子的生态生理活动直接受环境温度影 响(王培潮, 1966), 雄性性腺活动亦受温度季节 变化的影响。3-4月份的气温(15-20 左右) 可能最适合睾丸生精活动,此时睾丸平均重量和体 积达到全年最高值(图2A、B)。5-6月份气温持 续升高,7月份达全年最高点(图1),对应月份睾 丸重量和体积迅速减小并在7月份达到最低谷。由 此结果推测,高温抑制睾丸生精活动。8月份气温 开始缓慢下降,9月份以后睾丸开始复苏,至越冬 前(11月)睾丸重量和体积呈持续上升趋势。因 此,中国石龙子雄性性腺复苏发生在9月以后,睾 丸在动物越冬前已为其次年生精活动做好了准备。 3-4月份生精活动活跃期与野外的觅偶和交配活 动高峰期相一致。睾丸重量和体积的季节性变化反 映雄性中国石龙子的生殖年周期规律,其性腺的年 变化过程曲线呈"马鞍型", 这与组织学观察结果 一致。3月份时睾丸和附睾的重量之所以达全年最 高值,可能与睾丸中的生精活动和附睾为精子在其 中进一步发育成熟做好组织结构准备有关。输精管 重量在4月才达到最高值可能有两个原因:(1)附 睾中发育成熟的精子及分泌物输入输精管;(2)精 子及分泌物的输入刺激输精管结构的改变并导致增 重。6月份,睾丸和附睾中仅有少量精子,输精管 中未见精子,这表明能导致中国石龙子卵受精的交 配只能发生在 4-5 月份。

Cheng (1987) 将睾丸重量、生精活动、附性 器官之间的关系分为三种不同的类型:(1) 只有睾 丸重量出现周期性的循环,如疣尾蜥虎(*Hemi-dactylus f renatus*);(2) 附性器官的循环或多或少 与睾丸重量的周期性变化或生精活动相关,如蓬莱 草蜥(*Takydromus stejnegeri*);(3) 附性器官的周 期性变化与睾丸重量或生精活动无关,如台湾龙蜥 (*Japalura swinhonis*)。中国石龙子曲细精管直径 7 - 8月份最小,然后逐渐上升,在越冬开始的 11 月出现一个较低的峰值,管径仅略小于 3月。这一 现象提示,次年生精活动的结构准备在入冬前已趋 完成。曲细精管直径在 3月迅速达到最大值,腔内 出现成熟的精子。曲细经管 7 - 8月份直径最小, 生殖上皮仅存一层精原细胞,腔内已无精子。这一 结果表明,曲细精管管径的变化与睾丸、附睾和输 精管的变化有关,因此中国石龙子雄性生殖年周期 变化是属于上述的第二类种类型。

有鳞类爬行动物有宽广的繁殖对策谱,同域异 种之间繁殖策略的差异主要与物种在进化水平上的 遗传差异有关,而异域同种之间繁殖策略的差异主 要与各地环境变量的差异有关(Vitt, 1991; Howland, 1992; Vitt and Colli, 1994)。在影响蜥蜴生 精活动的环境因子中,温度很可能是一个主要因素 (Licht, 1967; Censky, 1995)。栖息于杭州的蓝尾 石龙子(Eumeces elegans)生精活动高峰与其同域 的中国石龙子一样发生在春季(王培潮, 1966), 而栖息于台湾中部高海拔地区的蓝尾石龙子生精活 动高峰发生在秋季(Huang, 1996);栖息于日本南 部的日本石龙子 (Eumeces atiscutatus) 生精活动 高峰出现在 8 月, 而栖息于日本中部的同种石龙子 生精活动高峰却在 4 月 (Hinoue, 1989)。因此, 雄性繁殖周期的地理变异既与不同地区年环境温变 模式的差异有关,也与物种的遗传差异有关 (James and Shine, 1985; Hinoue, 1989; Ota, 1994)。

附睾是雄性蜥蜴贮存精子的部位,对哺乳动物 精子成熟、获得运动能力等具有重要作用 (Robaire and Hermo, 1988)。附睾同样有助于爬行 类精子的成熟 (Depeiges and Dufaure, 1983; Esponda and Bedford, 1987),如胎生蜥蜴 (*Lacerta vivipara*)精子在通过附睾的过程中获得运动能 力 (Depeiges, 1984)。

中国石龙子雌体年产单窝卵(林植华、计翔, 2000;计翔、章朝华,2001)。王培潮(1966)认 为杭州中国石龙子卵泡萌发始于出眠之后的3月底 或4月初,与丽水中国石龙子卵泡萌发起始时间基 本一致。4月底到5月中旬,雌体陆续排卵,卵受 精后滞留在输卵管内,胚胎早期发育在输卵管内进 行。5月中旬到6月初为雌体的产卵期。因此,雌 雄中国石龙子配子的发生几乎同步,属于关联型繁 殖周期类型。

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## 图版说明

#### 中国石龙子睾丸、附睾和输精管显微结构

- 1.8月,曲细精管生殖上皮仅见精原细胞。
- 2.9月下旬,生殖上皮由分裂的精原细胞和精母细胞组成。
- 3.3月上旬,生殖上皮由精原细胞、精母细胞和精子细胞所构成。
- 4.4月中旬,曲细精管管腔中充满呈穗状排列的精子。
- 5.5月,生殖上皮萎缩,曲细精管中仍残留退化的精子。
- 6.7月,生殖上皮由精原细胞和支持细胞所构成。
- 7.4月下旬,附睾管中有大量的精子存在。
- 8.7月,附睾管中有无定形物(骨)存在,无精子。
- 9.4月下旬,输精管中有大量的精子存在,单层拄状上皮很厚。
- 10.7月,输精管的单层拄状上皮很薄,管腔中有无定形物 ( 1)。

i: 间质细胞。SC: 支持细胞。SI: 精原细胞。S2: 精母细胞。S3: 精子细胞。S: 精子。图中标尺均为 20 μm。

## Explanation of Plate

#### Histology of testis, epididymis and ductus differences in the Chinese skink Eumeces chinensis

- 1. Only spermatogonia could be found in the germinal epithelium of seminiferous tubules in August.
- 2. The germinal epithelium consisted of dividing spermatogonia and spermatocytes in later September.
- 3. Spermatogonia, spermatocytes, and spermatids could be found in the germinal epithelium in early March.
- 4. Numerous spermatozoa clustered in the lumen of seminiferous tubules in mid-April.
- 5. The germinal epithelium atrophied but regressed spermatogonia still existed in the seminiferous tubules in May.
- 6. The germinal epithelium was comprised of almost a single layer of spermatogonia and Sertoli cells in July.
- 7. A larger number of spermatozoa were present in the ductus epididymidis.
- 8. Amorphous material (arrow) but not spermatozoa could be found in the lumen of ductus epididymidis in July.
- 9. A large number of spermatozoa were present in the lumen of ductus deferens, but the simple columnar epithelium, which formed the wall of ductus deferens, was thick in later April.
- 10. The simple columnar epithelium forming the wall of ductus deferens was thin in July, and the lumen of ductus deferens was filled with amorphous material (arrow).
- i: Interstitial cells. SC: Stertoli cells. S1: Spermatogonia. S2: Spermatocytes. S3: Spermatids. S: Spermatozoa. Bars in the figure are all equal to 20  $\mu$ m.

## 胡健饶等:中国石龙子雄性生殖腺的年周期变化

HU Jian-Rao et al. : Annual variation in gonads of male Chinese skinks Eumeces chinensis



图版 Plate