# Ovarian Structural Features Reflecting Repeated Pregnancies and Parturitions in a Viviparous Scorpion, Liocheles australasiae

Authors: Yamazaki, Kazunori, and Makioka, Toshiki

Source: Zoological Science, 18(2) : 277-282

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.277

ZOOLOGICAL SCIENCE **18**: 277–282 (2001) 

# Ovarian Structural Features Reflecting Repeated Pregnancies and Parturitions in a Viviparous Scorpion, *Liocheles australasiae*

Kazunori Yamazaki* and Toshiki Makioka

*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan*

**ABSTRACT**—Eleven adult females of the viviparous scorpion, *Liocheles australasiae*, parthenogenetically repeated pregnancies and parturitions up to three times under the separate rearing. Only two of these females experienced the fourth parturition after the fourth pregnancy; one was dissected before the fifth pregnancy and the other early in the fifth pregnancy. In the ovaries of all these females, there were large empty ovarian diverticula, which had lost their embryos by the last parturition, and small ones as remnants of the previous parturitions. The number of all the empty ovarian diverticula in each female was roughly equal to the total number of her neonates. Even in the female in the fifth pregnancy, there were young ovarian diverticula containing oocytes other than the large ones containing embryos. The number of the young ovarian diverticula in the female seemed enough for one or two additional pregnancies, but further pregnancies and parturitions should become difficult because of deterioration of conditions for embryonic development under the long rearing and/or by the maternal ageing.

## INTRODUCTION

Most scorpions reproduce bisexually and only a few parthenogenetically. Embryos develop viviparously or ovoviviparously in the maternal ovary and youngs are born several months later (Polis and Sissom, 1990). In a few scorpion species, it has been known that a female can repeat pregnancies and parturitions under the separate rearing (Matthiesen, 1971; Polis and Sissom, 1990; Makioka, 1992a).

In a South American buthid scorpion, *Tityus serrulatus*, Matthiesen (1971) separately reared females to confirm thelytokous parthenogenesis and observed that a female parthenogenetically repeated parturitions four times and other two females three times, but he did not report ovarian structural features of the females after the repetition of pregnancies and parturitions. In adult females of some bisexual scorpions collected in the fields, Warburg and Rosenberg (1992, 1993, 1994), Warburg and Elias (1998), and Elias *et al*. (1999) estimated the number of parturitions, not only in the past, but also possible in the future, based upon differences in the ovarian structure, such as the sizes, shapes, and numbers of oocytes, embryos, and postpartum structures (ovarian structures remaining after the parturition), but these authors did not confirm the number of real times of pregnancies and parturitions under the rearing.

In adult females of the parthenogenetic ischnurid scorpion, *Liocheles australasiae*, separately reared in the laboratory, Makioka (1992a) described changes in the ovarian structural features during the repetition of three times of pregnancies and two intervening parturitions. Based upon the ovarian structural features reflecting past pregnancies and parturitions, Makioka (1992b) estimated the number of times of pregnancies and parturitions experienced in females of the species collected in the field up to the second parturition and the third pregnancy, but the ovarian structural features after the third pregnancy were left to be studied future.

In the present study, we have obtained adult females of *Liocheles australasiae* with repeated parturitions up to four times and pregnancies up to five times under the separate rearing and studied their ovarian structural features.

* Corresponding author: Tel. 0298-53-4910.
E-mail. s985621@ipe.tsukuba.ac.jp

## MATERIALS AND METHODS

A total of 413 females of *Liocheles australasiae* (Fabricius, 1775) (Ischnuridae) were collected from an all-female population in Iriomote Island, the Ryukyu Islands, Japan, in 1994. All these females were separately reared in glass vials (27 mm in diameter and 55 mm in height) with a piece of wet filter paper, at 28±1°C in an incubator, and fed with termites once a week. Juveniles born in the vials were separated into new glass vials as soon as possible after the first molt on their mothers' backs.

Thirty-three of juveniles became matured after five or rarely six molts in their vials and parthenogenetically repeated pregnancies and parturitions, but 22 of the 33 scorpions died before the third parturition. Eight females of the remaining 11 died after the third parturition, one before the fourth pregnancy and 7 in the fourth pregnancy. The other three females experienced the fourth parturition; one died before the fifth pregnancy, the other one was dissected early in the fifth pregnancy, and the remaining one is now in the fifth pregnancy. After all, 8 females after the third parturition and the other two after the fourth parturition were used as materials for the present study. The posthumous bodies were dissected as soon as possible, mostly within several hours after the death.

The ovaries of these 10 females mentioned above were removed in an isotonic physiological saline containing 0.7% NaCl and 0.3% $CaCl_2 \cdot 2H_2O$ to observe the gross anatomy and to count the number of the ovarian diverticula under a stereomicroscope and then fixed with Bouin's solution. The fixed ovaries were dehydrated in a graded ethanol and *n*-butyl alcohol series, embedded in paraffin, serially sectioned (5 μm thick), stained with Mayer's hematoxylin and eosin, and observed with a light microscope.

## RESULTS

### 1. Ovarian diverticula and neonates

The ovary consists of three longitudinal and four transverse ovarian tubes constructing a rough network. From these ovarian tubes are ventrally protruded a number of ovarian diverticula containing oocytes or embryos and those emptied by birth of their embryos (Fig. 1A and B). The former diverticula are larger in proportion to sizes of their oocytes or embryos and the latter smaller to the days after they released their embryos. Neither oogonia nor young oocytes were found in the wall of the ovarian tube, but all the oocytes were contained in their own ovarian diverticula (Figs. 2 and 3), as in adult ovaries before the third pregnancy (Makioka, 1992a), showing that no new ovarian diverticula containing oocytes are supplied during the repeated pregnancies.

Birth of neonates takes 10–20 hr. Neonates climb maternal body just after their birth and stay on the maternal back until they molt into the second instar juveniles to leave their mother about a week later. A few of the neonates sometimes failed to climb maternal body and fell onto the ground. Most of them could not climb up the mother again and soon died on the ground or were eaten by their mother. These dropouts were more frequent in the fourth parturition, in which the neonates were a little smaller and slenderer (about 5 mm in length and about 1 mm in width) than those in the earlier parturitions (about 7 mm in length and about 2 mm in width) in spite of their ordinary length of pregnancy (Fig. 4). Sometimes a few fully grown embryos failing to be born were retained in their diverticula, gradually degenerated and reduced in size. As a result, the total number of neonates counted in past parturitions were a little smaller than that of the empty ovarian diverticula.

### 2. Ovarian diverticula after the third parturition

Three types of ovarian diverticula were distinguished in 8 females after the third parturition; 1) small ovarian diverticula containing alecithal oocytes in growth phase (Fig. 3A and B), 2) large ovarian diverticula containing fully grown alecithal oocytes, mature eggs, or embryos developing and extremely growing (Fig. 3A), and 3) empty ovarian diverticula that lost embryos at the past three parturitions (Fig. 3A, B and C). The number of these ovarian diverticula was counted in the dissected ovaries (Table 1) and measured on the serial sections (Fig. 5A).

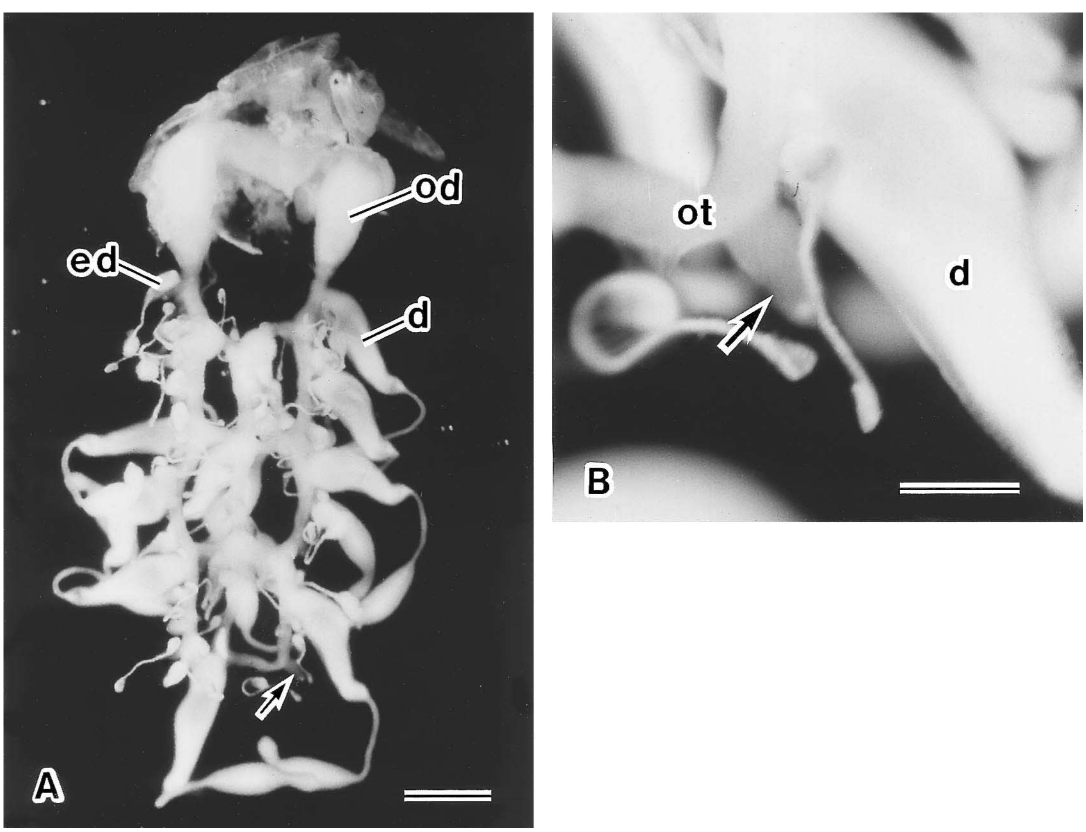


**Fig. 1.** (A) Ovary of *Liocheles australasiae* after the third parturition. Arrow shows small ovarian diverticulum containing oocyte. d, ovarian diverticulum containing embryo; ed, empty ovarian diverticulum; od, oviduct. Scale: 1 mm. (B) Enlargement of the small ovarian diverticulum containing oocyte (arrow). d, ovarian diverticulum containing embryo; ot, ovarian tube. Scale: 500 μm.

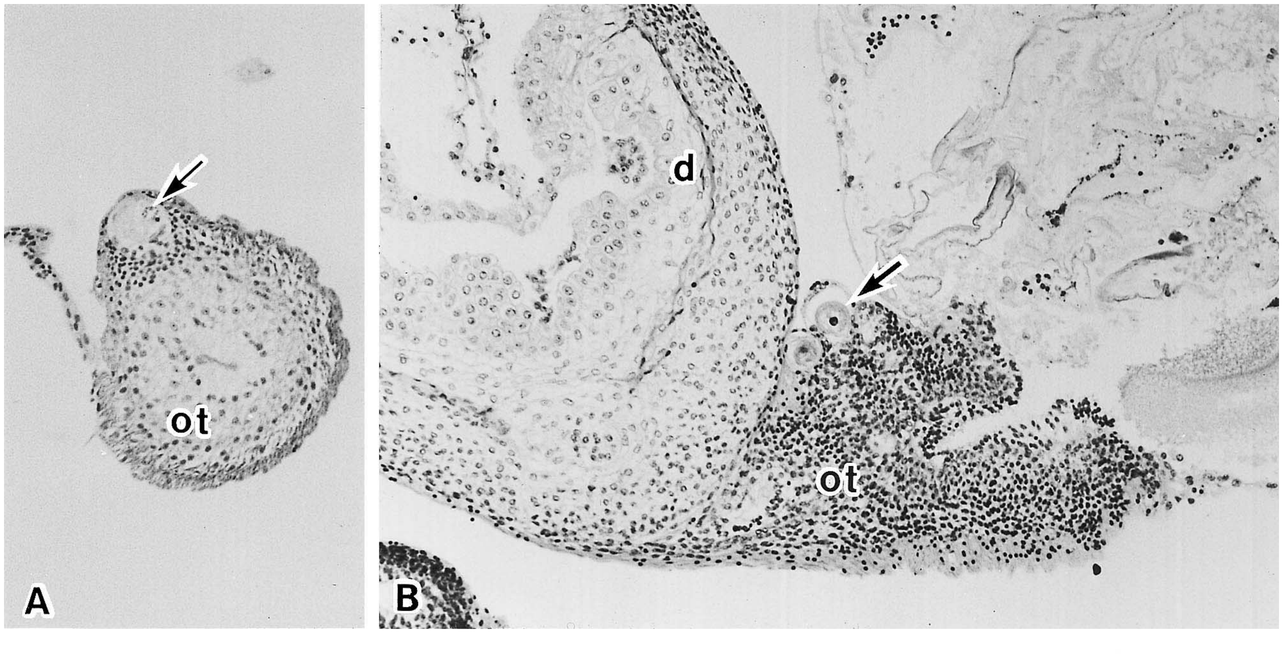


**Fig. 2.** Sections of the ovary of *Liocheles australasiae* after the third parturition. Scale: 100 μm. (A) Small ovarian diverticulum (arrow) containing an oocyte. ot, ovarian tube. (B) Small ovarian diverticulum (arrow) containing an oocyte and large ovarian diverticulum containing embryo (d). ot, ovarian tube.

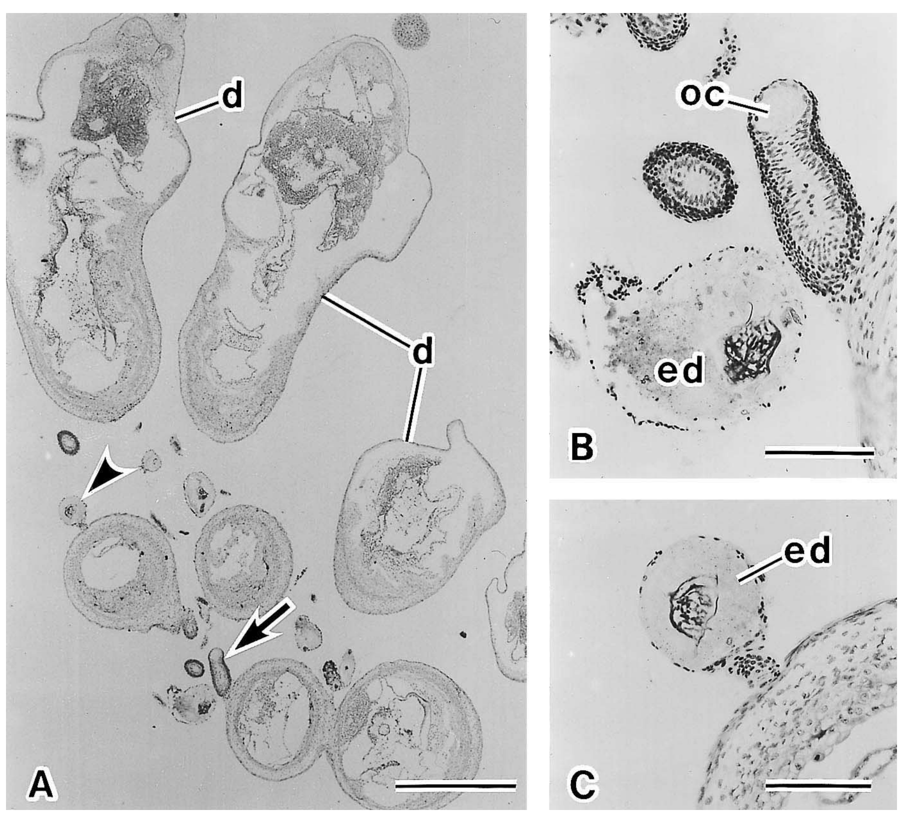


**Fig. 3.** Sections of the ovary of *Liocheles australasiae* after the third parturition. (A) Small ovarian diverticulum containing oocyte (arrow), large ovarian diverticulum containing embryo (d), and empty ovarian diverticulum (arrowhead). Scale: 500 μm (B) Enlargement of the small ovarian diverticulum containing oocyte (oc). ed, empty ovarian diverticulum sitting aside. Scale: 100 μm. (C) Enlargement of the empty ovarian diverticulum (ed). Scale: 100 μm.

**Fig. 4.** A neonate of *Liocheles australasiae* at the fourth parturition (left) and that at the first parturition (right). Scale: 1 mm.

The small ovarian diverticula with growing oocytes (25–50 μm in diameter) near their tips varied in size continuously (60–225 μm in length and 50–70 μm in width), but nevertheless the larger ones were regarded as a stock for the nearer pregnancy after next or the subsequent pregnancies. Each small ovarian diverticulum was a fine tubule branched from the ovarian tube. Its wall consisted of two layers of epithelia, continuing with those of the ovarian tube. It closed at the tip, encapsulating a growing oocyte surrounded by a layer of follicle epithelium. On the other hand, the larger ovarian diverticula containing fully grown oocytes (about 50 μm in diameter) or embryos for the next pregnancy or parturition, about 20 in number, were uniform in size and in growth or developmental stage. At the tip of each larger ovarian diverticulum containing an early embryo, there developed a narrow cord-shaped appendix for nourishing embryo (Polis and Sissom, 1990). Early embryos staying near the tips of their ovarian diverticula then rapidly grew, filling and extremely expanding their diverticula. Just before the parturition, the maternal abdomen was full of the ovarian diverticula filled with fully developed embryos. The appendices, completed their nourishing role, were shrinking at the tips of the largest ovarian diverticula.

The empty ovarian diverticula with small but distinct appendices on their tips, gradually decreasing in size, were divided into three size groups, small, medium, and large, supported by a rough correspondence with the numbers of neonates at the past three parturitions, 15, 15, and 13, respectively, in a female (Female 5 in Table 1). Their maximum widths were distributed in an ovary into 68–128 μm in the small, 138–157 μm in the medium, and 163–225 μm in the large group, respectively (Fig. 5A).

### 3. Ovarian diverticula after the fourth parturition

Ovarian diverticula in a female died soon after the fourth parturition, before the fifth pregnancy, were counted in the dissected ovary as follows; 1) 30 ovarian diverticula containing various sizes of oocytes, from the smallest to the largest, 2) 11 large empty ovarian diverticula that lost embryos by the fourth parturition, and 3) 45 small empty ones that lost the embryos by the past (before the fourth) parturitions (Female 1 in Table 2).

Ovarian diverticula in another female dissected early in the fifth pregnancy were counted in the dissected ovary as follows; 1) 19 small ovarian diverticula containing various sizes of oocytes, 2) 9 large ovarian diverticula containing early embryos, 3) 11 large empty ovarian diverticula that lost the embryos by the fourth parturition, and 4) 47 small empty ones that lost the embryos by the past (before the fourth) parturitions (Female 2 in Table 2).

The number of the small ovarian diverticula in the females after the fourth parturition were smaller than that in the females after the third parturition (Table 1 and 2). In the female before the fifth pregnancy, there were only the small ovarian diverticula containing various sizes of growing oocytes, which did not reach the final size yet (Female 1 in Table 2).

In these two females after the fourth parturition, the number of the large empty ovarian diverticula nearly corresponded to that of the neonates born at the fourth parturition (Table 2). The small empty ones were measured on the serial sections. They were divided into three size groups, small, medium, and large, supported by a correspondence with the numbers of

**Table 1.** The number of ovarian diverticula in eight females after the third parturition.

| Females | Ovarian diverticula with growing oocytes | Ovarian diverticula with fully grown oocytes or embryos | Empty ovarian diverticula | Embryos failed to be born | Total number |
|---|---|---|---|---|---|
| 1 | 26 | 12 | 42 | 3 | 83 |
| 2 | 19 | 12 | 43 | 0 | 74 |
| 3 | 31 | 11 | 38 | 4 | 84 |
| 4 | 32 | 15 | 40 | 8 | 95 |
| 5 | 21 | 15 | 43 | 0 | 79 |
| 6 | 43 | 12 | 22 | 7 | 84 |
| 7 | 31 | 4 | 15 | 15 | 65 |
| 8 | 39 | 16 | 19 | 10 | 84 |

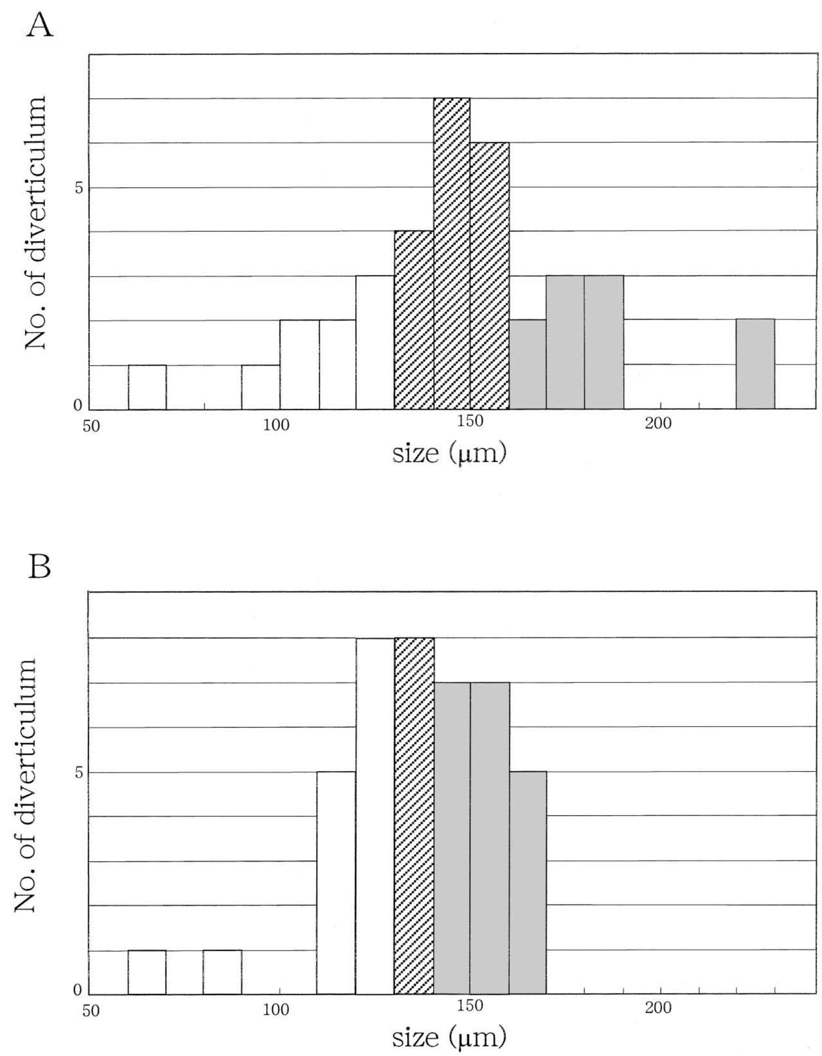


**Fig. 5.** Size variations in maximum width of empty ovarian diverticula (e.o.d.) of *Liocheles australasiae*, measured on serial sections. The open, shaded, and solid bars show e.o.d. after the first, second, and third parturitions, respectively. The number of e.o.d. roughly corresponds to that of neonates at each parturition. (A) Thirty six e.o.d. 161 days after the third parturition of Female 5 in Table 1. Seven e.o.d. were lost during the preparation of sections. (B) Forty two e.o.d., other than 11 extremely large e.o.d. (500–650 μm), just after the fourth parturition of Female 2 in Table 2. Five e.o.d. were lost during the preparation of sections.

**Table 2.** The number of ovarian diverticula in two females after the fourth parturition.

| Females | Ovarian diverticula with growing oocytes | Ovarian diverticula with fully grown oocytes or embryos | Empty ovarian diverticula | Embryos failed to be born | Total number |
|---|---|---|---|---|---|
| 1 | 30 | 0 | 56 | 3 | 89 |
| 2 | 19 | 9 | 58 | 1 | 87 |

neonates at the first, second, and third parturition, 11, 5, and 15, respectively, in a female (Female 2 in Table 2). Their maximum widths in an ovary ranged 68–126 μm, 130–138 μm, and 143–170 μm, respectively (Fig. 5B).

## DISCUSSION

### 1) Frequency of the past parturitions

It is useful to study ovarian structural features in adult female scorpions collected in the fields for estimating number of the past parturitions. The features should be compared with those in females, in which the past parturitions are recorded. However, it is not so easy to observe repeated parturitions in female scorpions, not only in the field, but also in the laboratory, and therefore, there are only a few studies with the confirmation of real number of times of parturitions (Matthiesen, 1962, 1971; Makioka, 1992b).

In adult females of *Liocheles australasiae*, separately reared in the laboratory, Makioka (1992a) observed that the ovaries before the first parturition have no empty ovarian diverticula, those after the first and before the second parturition have only large ones, and those after the second parturition have large and small ones, but he could not obtain females repeated parturitions three or more times. Subsequently, in adult females of *L. australasiae* collected in the field, Makioka (1992b) found ovaries without empty ovarian diverticula, those with only large ones, and those with large and small ones, and regarded them as those before the first parturition, those after the first and before the second parturition, and those after the second parturition, respectively, based on the results in the separate rearing (Makioka, 1992a). At the same time, he also discussed that, if the empty ovarian diverticula at the early parturitions have degenerated and disappeared in order, leaving only the last two types, ovaries with large and small empty ovarian diverticula in the field females may possibly include ovaries after the third parturition or after any of the subsequent parturitions (Makioka, 1992b). To verify the possibility, it was necessary to observe ovaries actually after the third parturition or after any of the subsequent parturitions.

In the present study, ovaries after the third and the fourth parturition and in the early fifth pregnancy were observed in the females of *Liocheles australasiae* separately reared in the laboratory. The large empty ovarian diverticula, remnants of the last parturition, were easily distinguished from the small ones by their sizes and shapes. The division into size groups of the small empty ones gradually decreasing in size was usually difficult, but sometimes supported by the numbers of neonates in the corresponding parturitions. The total number of the large and small empty ovarian diverticula were nearly equivalent to that of the neonates, showing that all the empty ovarian diverticula including those of the first parturition remain, not disappeared, even in the ovary in the early fifth pregnancy and that the number of the size groups of empty ovarian diverticula corresponds to the number of the past parturitions at least before up to the early fifth pregnancy.

**2) Expected number of the future parturitions**

In females used in the present study, a number of ovarian diverticula containing oocytes of various sizes were retained possibly for the future pregnancies in some ovaries after the third parturition and in the fourth pregnancy, as well as in the ovaries before the third parturition (Makioka, 1992a). Also in an ovary soon after the fourth parturition, there were 30 ovarian diverticula containing oocytes of various sizes possibly for the fifth and the subsequent pregnancies (Female 1 in Table 2). Moreover, in an ovary in the early fifth pregnancy, there were 19 ovarian diverticula containing oocytes of various sizes possibly for the sixth and the subsequent pregnancies, other than 9 large ovarian diverticula containing embryos (Female 2 in Table 2). As the number of neonates or their postpartum empty ovarian diverticula at each parturition is about 20 or less, it should be simply calculated that even the ovaries after the fourth parturition keep the capacity of additional pregnancies approximately up to twice.

Under the separate rearing in the present study, females after the third and the fourth parturition seemed easily to die, compared with those of the approximately same age but still staying in earlier adult stage. Furthermore, most of the neonates at the fourth parturition were smaller and slenderer than those at the earlier partutitions, even after the ordinary pregnancy time, and died within a few days after their birth. These facts suggest that the long laboratory rearing and/or the maternal ageing may have caused worsening of conditions for embryonic development. Under better rearing conditions or in the fields, females may possibly repeat one or two additional pregnancies and parturitions.

## ACKNOWLEDGMENTS

We are grateful to Dr. Kensuke Yahata, Dr. Koji Tojo, and Mr. Takao Kuwada, University of Tsukuba, Dr. Hitoshi Ando, Tsurumi University, and Dr. Kyosuke Ikuta, Kochi University of Technology, for their helpful discussions during the present study. We also thank Mr. Yuji Takayama, Mrs. Taeko Suzuki, Mrs. Keiko Kaya, Mrs. Hiroko Yahata, Mr. Nobuaki Kobayashi and Miss Hisako Shimada, University of Tsukuba, and Mr. Yasuhiro Nomoto, University of Tokyo, for their kind help in collecting and rearing specimens.

## REFERENCES

Elias R, Warburg MR, Rosenberg M (1999) Seasonal changes in the ovariuterus of female *Scorpio maurus fuscus* (Scorpiones; Scorpionidae), and the effect of higher temperature. Int J Insect Morphol Embryol 28: 337–348

Makioka T (1992a) Reproductive biology of the viviparous scorpion, *Liocheles australasiae* (Fabricius) (Arachnida, Scorpiones, Ischnuridae). II. Repeated pregnancies in virgins. Inv Repr Devel 21: 161–167

Makioka T (1992b) Reproductive biology of the viviparous scorpion, *Liocheles australasiae* (Fabricius) (Arachnida, Scorpiones, Ischnuridae). III. Structural types and functional phases of the adult ovary. Inv Repr Devel 21: 207–214

Matthiesen FA (1962) Parthenogenesis in scorpions. Evolution 16: 255–256

Matthiesen FA (1971) The breeding of *Tityus serrulatus* Lutz & Mello, 1927, in captivity (Scorpions, Buthidae). Rev Bras de Pesquisas Méd e Biol 4: 299–300

Polis GA, Sissom WD (1990) Life history. In “The biology of scorpions” Ed by GA Polis, Stanford University Press, Stanford California, pp 161–223

Warburg MR, Elias R (1998) The reproductive potential and strategy of *Scorpio maurus fuscus* (Scorpiones: Scorpionidae): anatomical clues in the ovariuterus. J Zool Lond 246: 29–37

Warburg MR, Rosenberg M (1992) The reproductive system of a scorpion, *Nebo hierichonticus* (Simon) (Scorpiones; Diplocentridae). Int J Insect Morphol Embryol 21: 365–368

Warburg MR, Rosenberg M (1993) The female reproductive system in *Scorpio maurus fuscus* (Scorpiones: Scorpionidae). Israel J Zool 39: 23–27

Warburg MR, Rosenberg M (1994) The female reproductive system of the eastern Australian scorpion. Tiss Cell 26: 779–783

(Received August 28, 2000 / Accepted October 17, 2000)