

## Gametogenesis in the leech *Asiaticobdella buntonensis* (Meyer, 1951) (Hirudinidae)

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Received 20 December 1991; accepted 20 May 1992

The gametogenic cycle of the leech, *Asiaticobdella buntonensis* from two localities was investigated and found to be simultaneously hermaphroditic. Spermatogenesis reached a state of maturation before oogenesis in a cycle of summer reproductive maturation and winter quiescence comparable to related Hirudinidae. Leeches collected from the more northerly, warmer water bodies on the Makhatini Flats (32°08'E / 27°27'S) were similar, although there is some evidence that their gametes matured earlier and remained in a mature condition longer than those collected from the more temperate collection site at Happy Valley Nature Reserve, Durban (31°00'E / 30°00'S). An understanding of the gametogenic cycle of this potential medicinal leech under natural conditions, provides an index against which the effects of manipulating laboratory conditions on mass rearing can be inferred.

Die gametogeniese siklus van die bloedsuier *Asiaticobdella buntonensis* afkomstig van twee streke is ondersoek en daar is gevind dat dit gelyktydige hermafroditisme vertoon. Oögenese word vooraf gegaan deur spermatogenese in 'n siklus van somer reprodutiewe rypwording en 'n winter rusperiode, vergelykbaar met verwante Hirudinidae. Bloedsuiers wat van die meer noordelike, warmer waterbronne op die Makhatinivlakte (32°08'E / 27°27'S) versamel is, word vroeër ryp en bly langer reprodutief aktief as die van die meer gematigde versamelplek by Happy Valley Natuurreserveat, Durban (31°00'E / 30°00'S). Kennis van die gametogeniese siklus van hierdie bloedsuier onder natuurlike toestande, verskaf die agtergrond waarteen manipulerings van laboratoriumtoestande vir massateling onderneem kan word.

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Leeches are invariably recorded as sequential protandrous hermaphrodites (Magadorn 1966, 1969; Olive & Clark 1978; Sawyer 1986; *inter alia*) although, as Davies & Singhal (1988) caution, the '... evidence for protandry rather than sequential cosexuality [in leeches] is rather scanty because investigators have either inferred the type of hermaphroditism while investigating other phenomena, or did not fully understand the differences between hermaphrodite types'. Studies of leech reproduction have yielded several variations on the hermaphroditic theme, particularly the phenomenon of sequential hermaphroditism. Kulkarni, Hanumante & Nagubhushanam (1978) ascribe a protandrous hermaphroditic reproductive cycle to *Poecilobdella viridis* (Moore 1927) (Hirudinariinae). However, their investigation of its gametogenesis, with the onset of spermatogenesis in February and oogenesis in March and subsequent co-occurrence of both activities until the start of winter reproductive quiescence in August, is more suggestive of simultaneous hermaphroditism; the lag between the onset of spermatogenesis and oogenesis being too slight and the subsequent overlap in co-occurrence too substantial to be classified as sequential cosexuality [*sensu* Davies & Singhal (1988)]. Similarly, Sawyer, Lepont, Stuart & Kramer (1981) investigated reproductive activity in *Haementaria ghilianii* (Filippi 1849) (Haementeriinae), which Sawyer (1986) describes as protandrous. This species has the capacity to produce and implant spermatophores while the female system is mature. Thus, although sequentially produced, the gametes are both

used within the same season, suggestive of sequential co-sexuality, not protandry.

Of the haematophagous Hirudiniformes, members of the genera *Hirudo* (Hirudininae) and *Poecilobdella* (Hirudinariinae) have been the subject of gametogenic investigation. Studies on *H. medicinalis* (Linnaeus 1758) (Hagadorn 1966, 1969, 1970; Malecha 1970a, 1970b; *inter alia*) and *P. viridis* (Hanumante & Kulkarni 1978; Kulkarni *et al.* 1978) have dealt with aspects of the seasonality, hormonal control and abiotic manipulation of gametogenesis. Their results reveal an annual cycle, controlled by a gonadotrophic factor released from the neurosecretory cells, the activity of which is positively correlated with increases in temperature and photoperiod.

At the cellular level, gametogenesis commences with gametes arising from the germinal epithelium of the ovi- or testisacs. The presumptive gametes are released into these coelomic sacs where they divide mitotically, but remain connected to a cytophore. In the male system, six synchronous mitotic divisions result in a cluster of 64 spermatogonia. In spermatogenesis all cells develop into spermatozoa, as opposed to oogenesis where one of the cells matures at the expense of the remaining nurse cells. Spermatocytes and oocytes then undergo meiosis. This first meiotic division results in 128 primary spermatocytes. After a second reductional division each cluster comprises 256 spermatocytes. The spermatocytes develop into mature spermatozoa, detach from the cytophore and remain in bundles held together by glandular secretions (Sawyer 1986).

The investigation into aspects of gametogenesis of *A. buntionensis* was undertaken since southern hemisphere Hirudinidae have not been examined in this way. In terms of the broader context of the present study it was useful to determine the nature of the gametogenic cycle of this potential medicinal leech so as to facilitate its breeding under laboratory conditions by providing a natural standard against which the effects of manipulation of abiotic factors under mass-rearing conditions could be inferred.

### Materials and Methods

Leeches were collected from two study sites, Happy Valley Nature Reserve, Durban (31°00'E / 30°00'S, average maximum and minimum ambient temperatures over the study period, obtained from the Louis Botha Airport were 25,2°C and 17,1°C respectively) and from water bodies on the more northerly Makhatini Flats (32°08'E / 27°27'S, average maximum and minimum temperatures, 28,4°C and 16,3°C respectively, obtained from the Makhatini Research Station). In the case of the more southerly study site, leeches were collected every month for the period July 1989–July 1990. Data from the Makhatini Flats cover the same period but were not available for the months of July, October, December, March and May. Oogenic data from Happy Valley for May and from the Makhatini Flats for April are omitted as poor histological procedure precluded enumeration.

Ten leeches from both areas were anaesthetized in carbonated water, weighed and then killed by freezing. The

described process was performed on a monthly basis, within two days of capture. Initially, two anterior and two posterior pairs of testes were dissected out, along with the ovaries, in order to establish whether the rate of spermatogenesis differed along the length of the body. No difference existed during the period July–September 1989 and only the anterior pairs were subsequently processed.

The testi- and ovisacs were fixed in Bouin's fixative, wax embedded, sectioned at 7 µm and stained in haematoxylin and eosin. Maturation changes were determined by the documentation of the proportional fluctuation of categories of spermatogenic and oogenic development. Spermatogenic stages (Figure 1) were categorized after Sawyer (1986) but expanded to include sperm bundles released from the cytophore [*sensu* Stage 5 and 6 of Davies & Singhal (1988) combined]. Spermatogenic stages enumerated were:

- Stage 1. Individual cells, or small isogenic groups of cells, cytophore not visible.
- Stage 2. Clusters of less than 32 large cells around a small cytophore.
- Stage 3. Larger clusters of cells with less abundant cytoplasm around a more prominent cytophore.
- Stage 4. Mature / maturing spermatids, either remaining attached to, or released from, the cytophore.

Oocytes were classified into three stages. Stage 1 consisted of undifferentiated cells in the ovarian strand; in Stage 2, the prospective ova were noticeably larger than the remaining cells in the ovarian strands; while Stage 3 included those

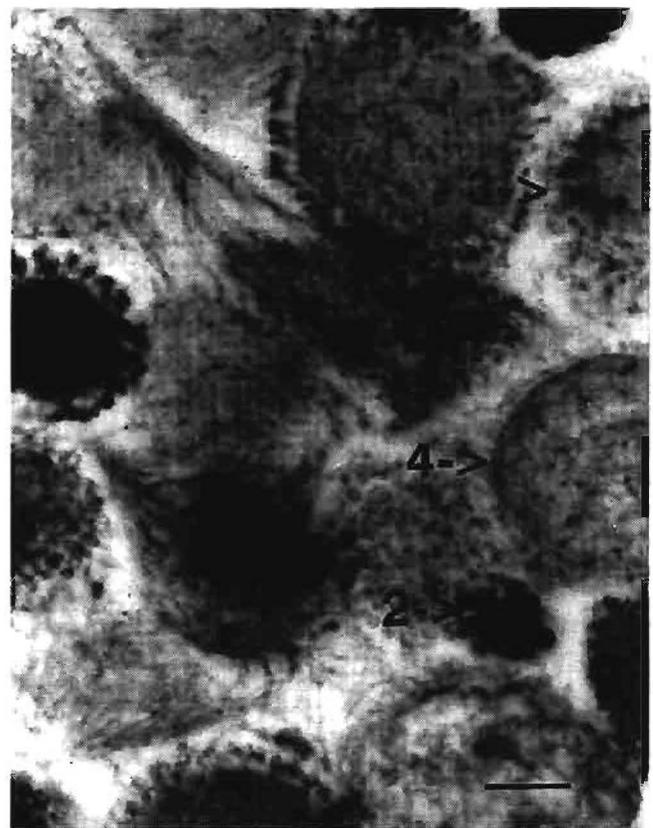
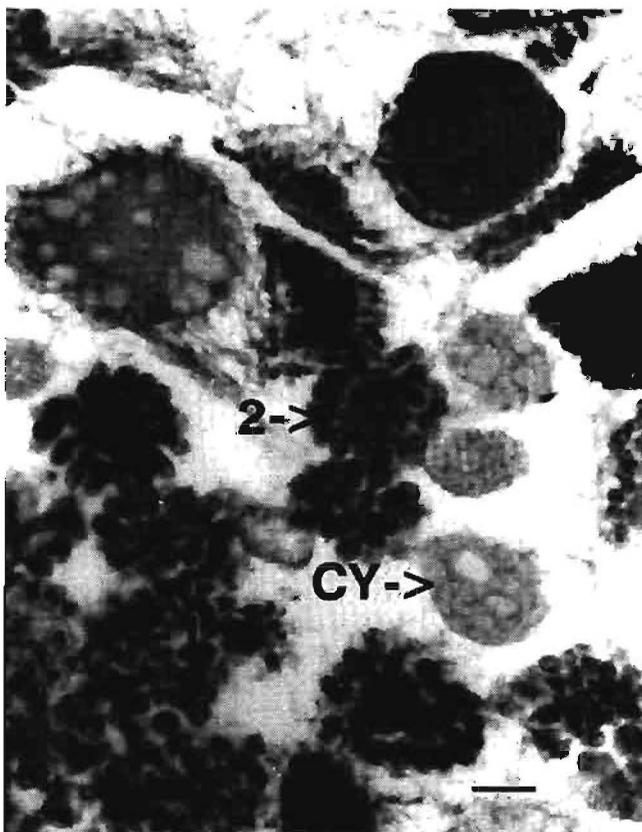


Figure 1 Spermatogenic stages of *Asiaticobdella buntionensis*. 1.1 TS through a maturing testisac showing early sperm cluster developmental Stages 1 and 2 as well as remaining cytophores (CY) from which spermatocytes have detached (Bar = 20 µm.) 1.2 TS through a mature testisac showing a predominance of Stage 4 sperm clusters together with Stages 2 and 3. (Bar = 20 µm.)

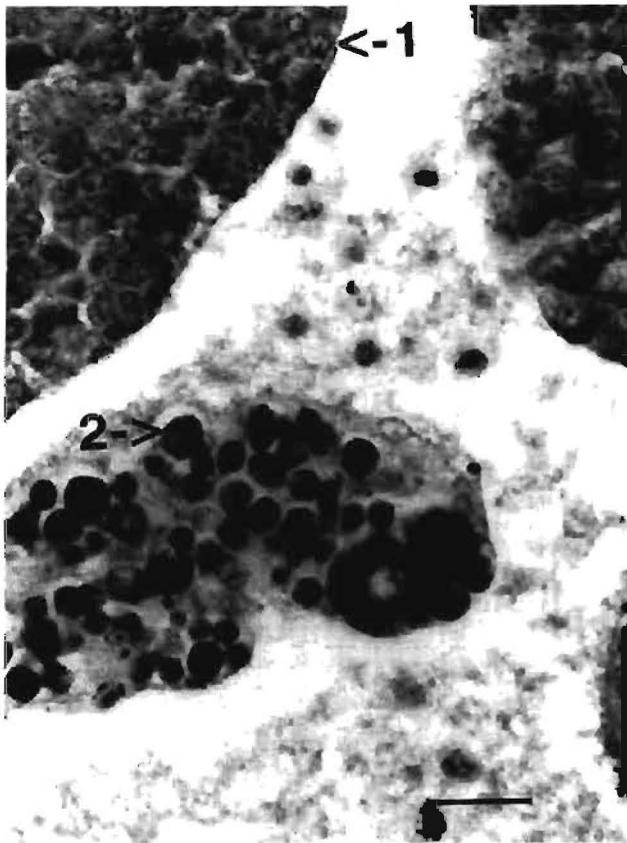


Figure 2 Oogenic stages in *Asiatocobdella bunttonensis*. 2.1 TS through a maturing ovarian strand showing Stage 1 and 2 oocytes. (Bar = 20  $\mu$ m.) 2.2 TS through a mature ovary showing mature oocytes (3) as well as undifferentiated cells (1) (Bar = 20  $\mu$ m.)

ovaries containing mature / maturing oocytes which had accumulated yolk [*sensu* Davies & Singhal (1988)] (Figure 2).

The percentages of spermatogenic stages were determined by counting in excess of 200 cells or clusters at 40 $\times$  magnification, which fell within the field of a photographic frame of a microscope eyepiece. Sperm clusters that were less than 50% included in the counting frame were not enumerated. The counting frame was always positioned so that one corner touched the periphery of the testicular contents in order to ensure an equal chance of including

Stage 1 sperm clusters which had recently germinated from, and were often located close to, the germinal epithelium. Ovaries were classified according to the most mature oocytes present.

### Results and Discussion

The seasonal fluctuations of the relative proportions of the four maturational stages of spermatogenesis of the Happy Valley population are depicted in Figure 3.1, while the data for the Makhatini Flats are presented in Figure 3.2. With

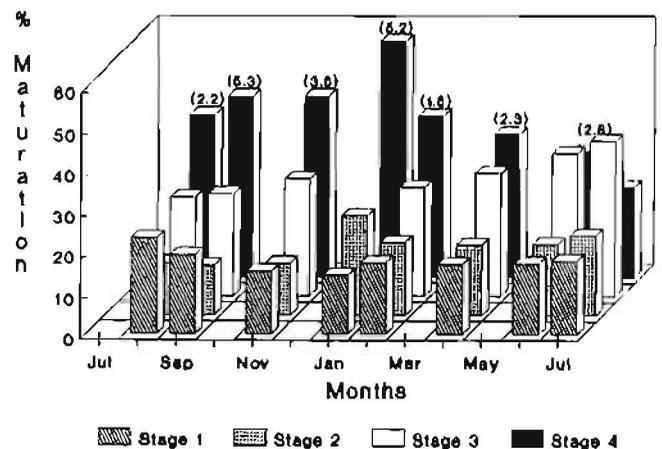
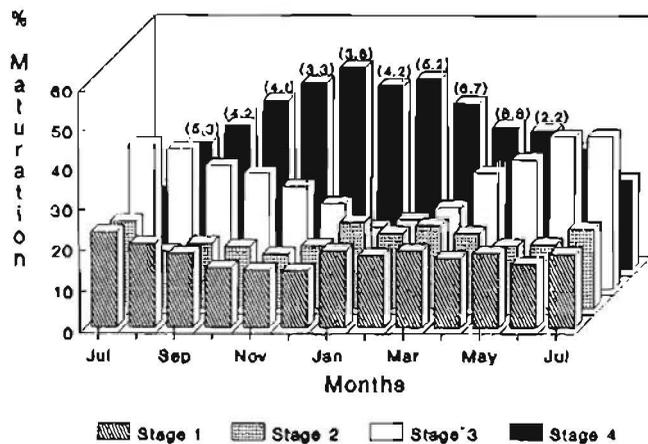
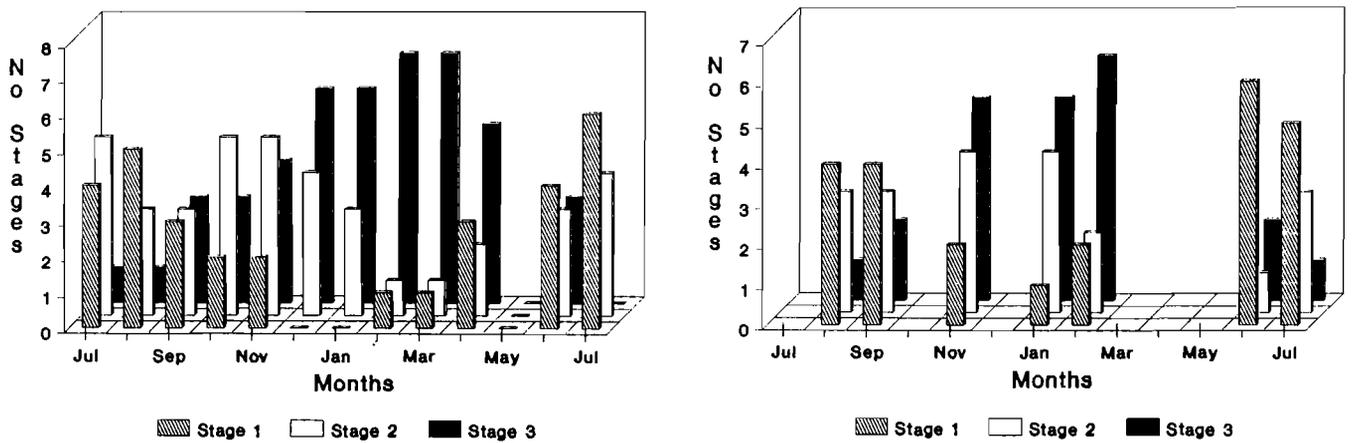


Figure 3 The spermatogenic cycle of *Asiatocobdella bunttonensis* for the period July 1989–July 1990. ( ) Standard error of the mean % of Stage 4 sperm clusters. 3.1 The spermatogenic cycle of leeches collected from Happy Valley Nature Reserve. 3.2 The spermatogenic cycle of leeches collected from the Makhatini Flats.



**Figure 4** The oogenic cycle of *Asiaticobdella buntonensis* for the period July 1989–July 1990. 4.1 The oogenic cycle of leeches collected from Happy Valley Nature Reserve. 4.2 The oogenic cycle of leeches collected from the Makhatini Flats.

proportional enumeration of a fixed number of cells or clusters, the absence or abundance of one stage may falsely influence the recorded number of another. Thus in the spermatogenic data the documented proportional fluctuations of the smaller Stage 1 and 2 classes are strongly influenced by the numbers of the larger Stage 3 and 4 sperm clusters. Because of this, and the fact that the stages of early development co-occur with Stages 3 and 4 throughout spermatogenesis, the spermatogenic cycle is indicated most clearly by the number of mature stages. This problem also influenced the adoption of the above spermatogenic stages; maturation trends being clearer within only four categories than in those with several subdivisions and as many as six stages.

The pattern of both the spermatogenic and the oogenic pathways (Figures 3 and 4) is similar to that of *Poecillobdella viridis* (Hanumante & Kulkarni 1978) except that the latter leech follows the northern hemisphere seasonal cycle. *A. buntonensis* follows an annual seasonal cycle, with a winter quiescence with few mature gametocytes progressing to maturity until mid-summer. In the more completely studied population at Happy Valley, Stage 4 sperm clusters predominated from November to February, while Stage 3 oocytes are most abundant from December until at least April. As in the case of *P. viridis*, the duration of the co-occurrence of both mature reproductive systems indicate simultaneous hermaphroditism and not sequential co-sexuality as the lag between the appearance of mature sperm and ovarian stages initially suggests.

Although incomplete, the data from the Makhatini Flats allow some comparison of the gametogenic pathways of the populations from the two study sites. The seasonality of both is very similar except for the occurrence of a significantly higher proportion (Student's *t* test) of Stage 4 sperm-clusters in the Makhatini Flats population in August and September (32,3% vs 39,8%;  $p < 0,05$  and 36,0% vs 43,7%;  $p < 0,05$ , respectively), indicative of an earlier onset of gametogenic activity in leeches from the warmer of the two study sites.

Photoperiod, temperature and nutritional state appear to be the most prominent factors influencing the onset and maintenance of reproductive activity in leeches which

correlate well with seasonal changes in abiotic conditions (Malecha 1970a; Hanumante & Kulkarni 1978; Kulkarni *et al.* 1978; Sawyer *et al.* 1981; *inter alia*). Invoking temperature as a major factor explaining the differences between the two study sites is confounded by the fact that although the Makhatini Flats is the warmer region in summer [average maximum and minimum for November 1989–April 1990, 29,5 and 19,8°C (mean, 24,6°C) vs 26,4 and 19,5°C (mean, 22,9°C) recorded for Happy Valley] its winter temperatures are less divergent, having a colder minimum average than Happy Valley [average maximum and minimum temperatures for the cooler months of the study May–October, 27,4 and 12,4°C (mean, 19,9°C) vs 23,7 and 13,9°C (mean 18,6°C)], the latter site enjoying the ameliorating effects of its proximity to the sea. However, the studies cited above, especially that of Hanumante & Kulkarni (1978) who showed that acclimation to a higher temperature for only 15 days influenced oocyte diameter and the numbers of mature sperm clusters in *P. viridis*, indicate that temperatures experienced during the onset and maintenance of reproductive activity would have the most influence on gametogenesis. Thus temperature either in isolation or combined with other abiotic factors such as photoperiod may account for the earlier production of mature gametes in the Makhatini Flats population. The exact nature of the stimuli initiating and maintaining gametogenesis in this leech will require laboratory investigation as concrete conclusions can best be drawn when off-season gametogenesis can be induced by manipulation of the implicated environmental conditions (Kulkarni *et al.* 1978).

#### Acknowledgements

The authors gratefully acknowledge the identification of *Asiaticobdella buntonensis* by Dr J.H. Oosthuizen (University of Pretoria) and thank Drs B.L.S. Sharp, D. le Sueur and C. Dettman and Mr D. Mthembu (Medical Research Council, Natal) for their assistance with the location and capture of leeches in Northern Zululand. Our thanks are extended to both the Natal Parks Board and the Kwazulu Bureau of Natural Resources for permission to collect leeches from water bodies under their jurisdiction.

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