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## One-Sided Ejaculation of Echidna Sperm Bundles

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**ABSTRACT:** We report for the first time an unusual ejaculatory mechanism in the short-beaked echidna in which each side of the bilaterally symmetrical, rosettelike glans penis is used alternately, with the other being shut down. This is unparalleled in mammals but is reminiscent of the use of hemipenes in squamate reptiles, providing further reproductive evidence of a sauropsidian lineage in the Monotremata. Further, we describe the occurrence of motile sperm bundles in ejaculated echidna semen and provide scanning electron micrographs of their morphology. Sperm bundling appears to confer increased sperm motility, which may provide the potential for sperm competition between males.

**Keywords:** short-beaked echidna, ejaculation, penis, sperm, Monotremata, sperm bundles, sperm competition.

Monotreme reproduction has been characterized as an unusual blend of reptilian and mammalian features, suggestive of a shared evolutionary history (Griffiths 1989; Temple-Smith and Grant 2001). Much of the fascination about monotreme reproduction has been centered on the egg-laying female, but clearly, the male also demonstrates

a range of unusual reproductive features, some of which support a close phylogenetic relationship with the reptiles. We present evidence to further support this by describing for the first time a strange form of ejaculatory behavior in the echidna, whereby only half the glans penis is used. We also describe ejaculated bundles of spermatozoa that swim cooperatively and discuss that sperm competition may be important in this species.

The short-beaked echidna (*Tachyglossus acelatus*; Monotremata) penis is used only for the passage of semen, with urination occurring directly into the cloaca via the urethral papillae (Augee et al. 2006). The glans penis is bifid, and each urethral portion bifurcates further into two soft epidermal rosettes. The urethrae terminate on the surfaces of the rosette in a series of openings similar to that of a showerhead. During the early stages of erection (fig. 1), the glans penis has a quadripartite anemone-like appearance (Griffiths 1999). How this unusual four-headed glans penis is used during copulation has been a long-standing mystery (Temple-Smith and Grant 2001; Jones et al. 2004).

In 2005, Currumbin Wildlife Sanctuary (Gold Coast, Australia) came into possession of a 17-year-old captive male echidna that had become habituated to human presence as part of an interactive public display. Zookeepers noted that, on handling, this animal would readily produce an erection. Over a period of 2 weeks, zookeepers at Currumbin Wildlife Sanctuary conditioned the echidna to develop an erection to the point where it would ejaculate. In preparation for semen collection, the echidna is placed in lateral recumbency on a clean surface of the floor of its enclosure. Using a closed fist, the zookeeper gently pushes his hand into the lower abdomen of the animal, at which time the echidna usually responds by pushing its cloaca up against the zookeeper's fist and developing an erection (video 1; fig. 1).

During the early stages of erection, the glans penis displays all four rosettes, but as the erection continues, two of the rosettes retract, leaving the remaining rosettes fully engorged and slightly rotated, to give the fully erect penis a symmetrical appearance (video 1; figs. 1, 2A). The penile morphology of the erect echidna penis is therefore, at this point, completely compatible with the anatomy of the cranial portion of the female's urogenital sinus. In this form,

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**Figure 1:** Erection and ejaculation resulting in semen collection in the short-beaked echidna. Note the progressive shutdown of the left side of the glans penis.

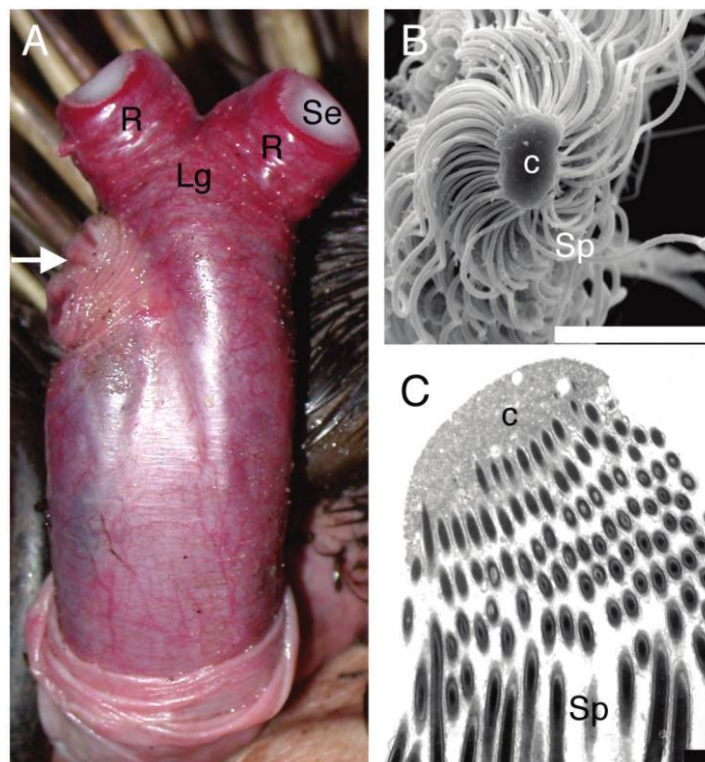
the engorged penis would appear to deliver the semen directly adjacent to the female's oviductal ostia. Ejaculation commences approximately 20 s after the penis has become fully erect. When erect, the penis was about one-quarter of the echidna's body length. Erection and ejaculation typically lasted between 10 and 15 min. During ejaculation, the semen pooled into the cups of the rosettes as a white viscous fluid (fig. 2A).

This highly unusual form of one-sided ejaculation is unlikely to be an abnormality associated with urethral blockage, as 10 consecutive observations of ejaculation revealed that the left and right sides of the glans were used alternately. Such ejaculatory behavior has never been described in a mammal, but it is reminiscent of the independent use of hemipenes in squamate reptile copulation (Gist 1999). It is difficult to know whether ejaculation is restricted to one side of the complete reproductive tract (epididymis + vas deferens), as is likely to be the case in the Squamata, or whether the differential use of the penis in the echidna occurs only at the level of the glans penis.

Once the echidna semen had pooled in the rosettes of the glans penis, it was possible to remove a small sample (approximately 20  $\mu\text{L}$ ) for examination. Five semen samples, all collected from the same animal during August 2005, were evaluated for their seminal characteristics by using phase contrast microscopy and were prepared for transmission and scanning electron microscopy. The pH of ejaculated semen was 7.4. When fixed in 10% buffered formalin, sperm typically dissociated from their bundles, and it was possible to determine sperm concentration, which ranged from  $1.0 \times 10^9$  to  $8.5 \times 10^9$  sperm  $\text{mL}^{-1}$  ( $n = 4$ ). Semen samples contained bundles of up to 100 spermatozoa that were joined at their apical extremity and were observed to swim progressively forward in a vigorous and co-ordinated pattern (video 2). While sperm bundles

in echidnas are known to form during epididymal maturation (Djakiew and Jones 1983) and have previously been observed to be highly motile in native epididymal fluid (Jones et al. 2004), this study provides the first observation of motile sperm bundles in undiluted ejaculated semen. While no estimates of sperm bundle velocity were calculated, spermatozoa in larger bundles showed greater progressive motility than single spermatozoa or smaller sperm bundles (video 2; Jones et al. 2004). The rostral tips of the sperm heads were joined by an electron-dense amorphous "cementing material" and were stacked tightly adjacent to each other, forming multiple spires about their spiral-shaped nuclei (fig. 2B, 2C).

There are several possible explanations for the occurrence of sperm bundles in echidna semen. Perhaps the most likely is that the formation of bundles is associated with the evolution of some form of postcoital sperm competition (Jones et al. 2004). This idea is supported by the comparatively large size of echidna testes, the high number of extragonadal sperm (Jones et al. 2004), and the observation that male echidnas can form mating trains of up to 11 animals behind an estrous female, with the dominant male in the front of the line (Rismiller and Seymour 1991; Augee et al. 2006). In addition, mating can be a prolonged event (30–180 min; Augee et al. 2006) in the echidna and may be associated with a copulatory tie (Rismiller and Seymour 1991); both these phenomena are likely to be mechanisms that exclude copulatory opportunity for male rivals. The limited development of or lack of accessory glands in the echidna (Augee et al. 2006) means that the female reproductive tract cannot be "swamped" by excessive seminal plasma, so, accordingly, a different strategy for sperm competition may have evolved in which sperm form and swim in bundles so as to facilitate rapid access to the oocyte. Another possibility is that the sperm bundle



**Figure 2:** A, Erect short-beaked echidna penis demonstrating one-sided retraction of the right side of the glans penis (arrow) and pooled semen in the two rosettes of the functional left side. B, C, Scanning and transmission electron micrographs of ejaculated sperm bundles (scale bars = 10  $\mu\text{m}$  and 500 nm, respectively). B, Apical view. C, Lateral aspect. c = "cement substance"; Lg = left glans penis; R = rosette; Se = semen; Sp = sperm. Video 1 (QuickTime; 5.96 MB) demonstrates erection behavior and ejaculation in the short-beaked echidna. Video 2 (QuickTime; 1.25 MB) shows phase contrast microscopy of motile sperm bundles in undiluted semen. Larger sperm bundles swim at a greater velocity than smaller bundles or individual spermatozoa.

is a mechanism for reducing premature capacitation of those sperm on the inner side of the bundle not directly exposed to the secretions of the female reproductive tract. And, further, the sperm bundle may be some form of strategy for storage of sperm in the female echidna's reproductive tract, similar to that found in most avian species (Williams 1999). The ability to collect echidna semen samples regularly will provide further material to help us better understand the benefits of bundle formation and other aspects of sperm physiology in this phylogenetically important taxon.

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