

A shot in the dark: same-sex sexual behaviour in a deep-sea squid

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Little is known about the reproductive habits of deep-living squids. Using remotely operated vehicles in the deep waters of the Monterey Submarine Canyon, we have found evidence of mating, i.e. implanted sperm packages, on similar body locations in males and females of the rarely seen mesopelagic squid *Octopoteuthis deletron*. Equivalent numbers of both sexes were found to have mated, indicating that male squid routinely and indiscriminately mate with both males and females. Most squid species are short-lived, semelparous (i.e. with a single, brief reproductive period) and promiscuous. In the deep, dark habitat where *O. deletron* lives, potential mates are few and far between. We suggest that same-sex mating behaviour by *O. deletron* is part of a reproductive strategy that maximizes success by inducing males to indiscriminately and swiftly inseminate every conspecific that they encounter.

Keywords: *Octopoteuthis deletron*; Cephalopoda; mating; spermatangia; same-sex sexual behaviour; deep-sea squid

1. INTRODUCTION

Squids are highly diverse and populous in the deep oceans, and they play a vital role in meso- and bathypelagic food webs [1]. The reproductive behaviour of deep-sea squids, however, remains unknown. The investigation of dead animals (i.e. from trawls and predator stomachs) has revealed clues that deep-sea squid mating behaviour is mechanistically different from that known in coastal squid [2–4]. Direct observations of mating behaviour of deep-sea squid are restricted to a single observation of a possible mating event [5]. The transition from indirect to directed methods, e.g. remotely operated vehicles (ROVs) [6] for exploration and research, has shed new light on our knowledge of deep-sea squid behaviour [7–9]. Based on ROV observations, we describe mating habits of *Octopoteuthis deletron*, a deep-sea squid from the Eastern Pacific, with hook-lined arms and a suite of photophores on the ventral side and arm-tips.

Males of the genus *Octopoteuthis* transfer spermatophores, complex structures containing millions of sperm, using a long terminal organ often referred to as a penis. During opposite-sex mating, spermatophores are deposited on the female, where they discharge sperm-containing sacs called spermatangia that implant

into the female's tissue. Empty spermatangia remain attached to a female's body and provide evidence of recent mating [2,4,10]. We found such evidence during *in situ* observations of *O. deletron* in both males and females. We report here for the first time in invertebrates that intraspecific same-sex sexual behaviour (SSB) [11] happens as frequently as opposite-sex sexual behaviour in this species.

2. MATERIAL AND METHODS

Our results are based on video footage of 108 specimens of *O. deletron*. Live observations were recorded by the Monterey Bay Aquarium Research Institute's ROVs between 1992 and 2011, mostly over the Monterey Submarine Canyon, CA, between 400 and 800 m depth. Each video sequence was reviewed, and frame-grabs were taken of specific body regions. The individual squid in these frame grabs were then examined for the presence of (i) implanted spermatangia (i.e. discharged spermatophores) that store sperm on a female's body until fertilization and (ii) rugose tissue, a deeply wrinkled semi-gelatinous tissue found on the anterior ventral third of the mantles of mature or maturing females (figure 1a). This tissue was recognizable in ROV video footage of an immature female as small as 100 mm mantle length (ML) that was subsequently collected and examined. We used the presence or absence of rugose mantle tissue to determine the sex of individuals in video footage. Each animal was visually divided into four ventral and four dorsal locations: the arms, head, mantle and fins, and the tail. Where possible, the number of spermatangia in a location was counted from frame grabs; if the frame grabs did not show enough detail to allow counts, only spermatangia presence was recorded. We physically examined, and determined ML and body mass (BM), and the sex and maturity stage of five individuals (two males and three females) collected during ROV dives and preserved in 5 per cent formalin. Observations on numerous mature male and female specimens from museum collections further supported the conclusion that rugose tissue was only present in female *O. deletron*. This sexually dimorphic character was previously reported also for *Octopoteuthis sicula* [10]. Spermatangia implanted in the skin of preserved specimens were counted, measured and photographed.

3. RESULTS

In total, 108 individual *O. deletron* were recorded on ROV video footage. Fifteen juveniles were seen, and for 54 individuals the footage was not clear enough to determine maturity stage, sex or the presence of spermatangia. We could differentiate between sexes in 39 animals: 19 females and 20 males, consistent with a 1 : 1 population sex ratio. Of these individuals, nine males and 10 females had groups of spermatangia in both dorsal and ventral body locations (figures 1,2). Males were as likely to be found mated as females were (Fisher's exact test: $p > 0.05$). Although *O. deletron* was observed in all months of the year, mated individuals were only encountered between June and December. Spermatangia had a bulbous proximal portion (approx. 1.5 mm long and approx. 0.8 mm wide) containing the sperm, and a distal, open-ended, trailing tube (2–4 mm long and 0.1 mm wide; figure 1b). Of the nine specimens on which we could count spermatangia from video footage: two males had 15 and 25 spermatangia, and seven females had spermatangia numbers ranging from 21 to 147. Spermatangia implantation occurred principally on the dorsal mantle and fins in both sexes, and was also common on the ventral arms of females (figure 2). A mature male *O. deletron* (ML 152 mm, BM 241 g) had 1646 spermatophores of approximately 14 mm length in its reproductive system.

4. DISCUSSION

SSB in marine invertebrates is very poorly known. SSB has been documented in the sequentially hermaphroditic

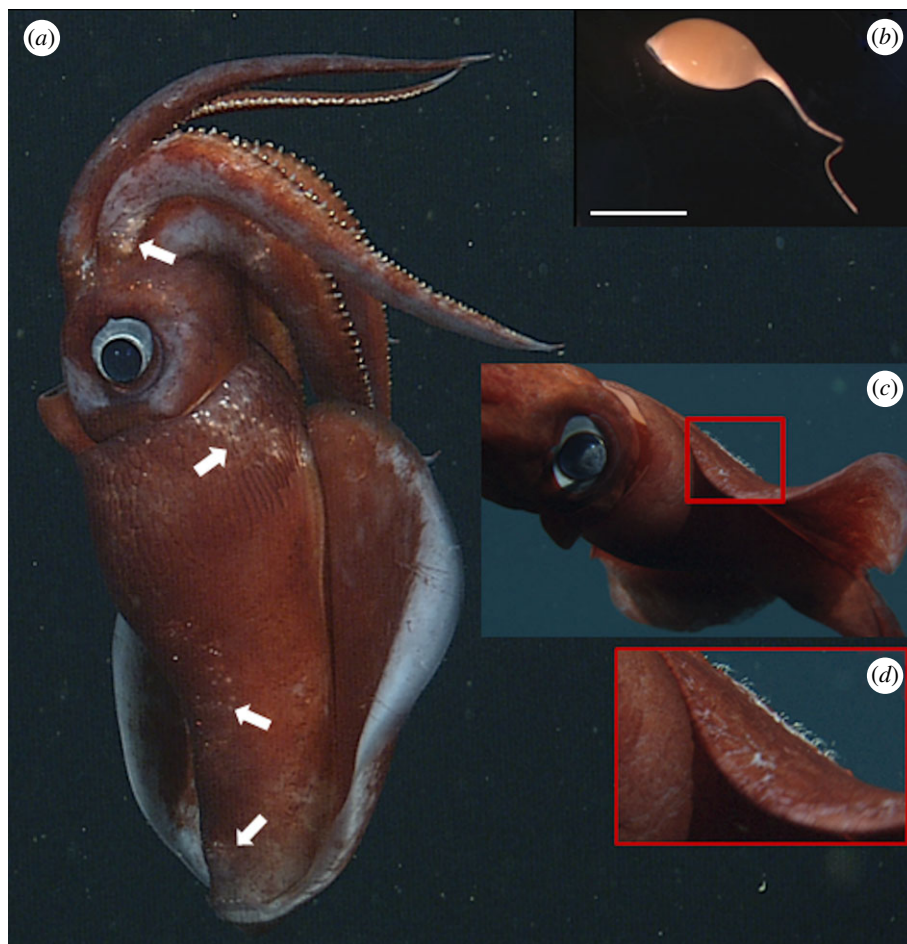


Figure 1. (a) A female *Octopoteuthis deletron* showing implanted spermatangia in the ventral arms, the ventral lateral mantle and the ventral mid and posterior mantle (arrows) and the characteristic rugose skin of the anterior mantle in females. (b) A spermatangium showing the bulbous proximal portion containing sperm, and the trailing open end. (c) A male *O. deletron* with implanted spermatangia on the dorsal mantle; the trailing ends are visible in profile. (d) Close-up of (c).

marine gastropod *Crepidula fornicata* [12] and although dissimilar to what we report here, SSB has been reported before in cephalopods. Two male deep-sea incirrate octopods of different species were observed in a copulation event at 2512 m depth [13]. In the ommastrephid squid *Illex coindetii*, among 179 examined males, one mated male was found [14]. This is the only record of a mated male ommastrephid in the literature. Since this squid family sustains enormous commercial fisheries, and biological examinations of trawl caught individuals are numerous, we can safely assume that male-to-male mating is a very rare phenomenon in this family.

In giant squid (*Architeuthis* sp.) from the Atlantic that were either collected by net, washed up on the shore or found floating on the sea surface, seven of 12 mature males had spermatangia implanted, mainly in their ventral arms [15–17]. Similarly, four trawled male *O. sicula* were found to have spermatangia implanted in their ventral arms [10]. In the absence of direct observation of undisturbed specimens, it is questionable if the spermatangia in these males were the result of SSB or were caused by trauma in the trawl, as pressure on the terminal organ induces release of spermatophores and subsequent implantation. Accidental self implantation during mating with a female is also a possibility.

The male *O. deletron* we observed had spermatangia in locations other than the ventral arms, in similar

locations to those of mated females. Because the spermatangia were in locations that are outside of the reach of their own terminal organs, we conclude that the spermatangia on these males were implanted by other males.

Although SSB has been documented in a variety of animals [11], including cephalopods [18], *O. deletron* is unique in that we found equal proportions of mated males and mated females. This suggests that SSB is as common as opposite-sex sexual behaviour in this species. The number of spermatophores produced by a male *O. deletron* (over 1600) far exceeds the number of spermatangia found on any individual after mating, indicating that *O. deletron* is capable of multiple mating events. While promiscuity is common in cephalopods [19], the risks and costs of same-sex mating raise the question of its apparent negative evolutionary value.

The sensory means by which squids find mates in the deep sea are unstudied. In the dark waters that adult *O. deletron* inhabit (400–800 m depth), downwelling sunlight cannot aid much in recognizing potential mates. Mesopelagic squid in general have highly developed eyes with visual acuity sufficient to allow the detection of point sources and patterns of bioluminescence [20]. The light-producing organs on the tips of *O. deletron*'s arms can be turned on and

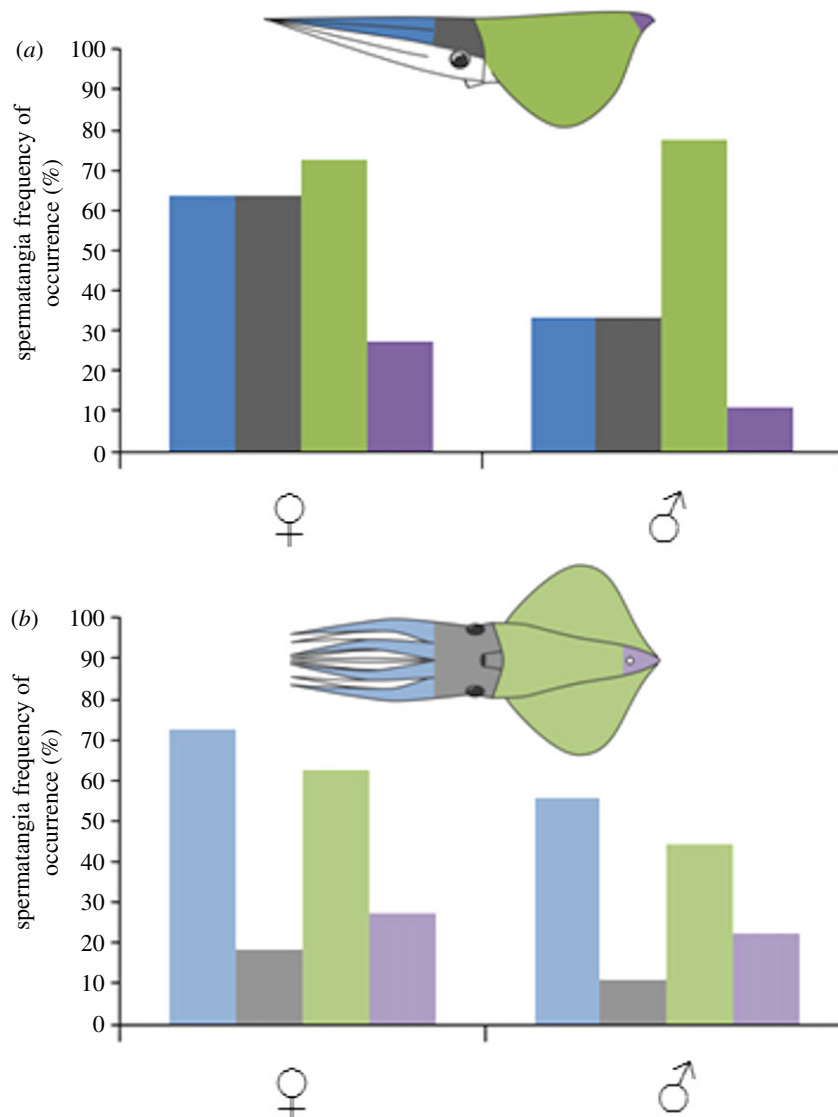


Figure 2. Frequency of occurrence of spermatangia present on the (a) dorsal and (b) ventral body of male and female *O. deletron*. The colours of the body parts correspond with the colours of the bars in the graph.

off rapidly and modulated by overlying chromatophores, either simultaneously or independently, and could potentially be used in gender recognition, as could the many other photophores they possess. Likewise, this species is capable of producing a wide variety of pigmentation patterns using the chromatophores on its body [7]. This capacity could also enhance communication by acting as fluctuating light shutters of the photophores, as in the arm-tips. However, in spite of the potential for recognizing mates and the relative availability of members of both sexes (a 1:1 sex ratio), we observed statistically indistinguishable proportions of mated males and females. We conclude, therefore, that male *O. deletron* mate non-selectively with any conspecific.

A failure to exhibit sex discrimination and the acceptance of same-sex mates are generally considered a recognition error with an attendant reproductive cost [12]. Squid typically have a single, brief reproductive period and then they die [19]. Mating in *O. deletron*, as in many other squids, is probably rapid, as spermatophores are quickly passed between partners and

spermatangia release follows soon thereafter [19]. Mature males and maturing mated females of *O. deletron* are of the same size and have only minor morphological differences. We have only observed them as solitary individuals. The combination of a solitary life, poor sex differentiation, the difficulty of locating a conspecific and the rapidity of the sexual encounter probably results in the observed high frequency of spermatangia-bearing males in this species. Apparently, the costs involved in losing sperm to another male are smaller than the costs of developing sex discrimination and courtship, or of not mating at all. This behaviour further exemplifies the 'live fast and die young' life strategy of many cephalopods [19].

We thank the David and Lucile Packard Foundation, The Netherlands Organization for Scientific Research (NWO) and the Royal Dutch Academy of Science (KNAW). We thank R. Sherlock, Drs R. E. Young, K. Osborn, D. Haydar, M. Vecchione and an anonymous referee for valuable comments on the manuscript. Also we would like to thank the people from MBARI's videolab and the pilots of the ROVs *Tiburón*, *Ventana* and *Doc Ricketts* and ships'

crews of the R/Vs *Point Lobos* and *Western Flyer*, as this study would not have been possible without their efforts.

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