THE ART OF *MATING*

by Eamon Durkan | 20 Dec 2023

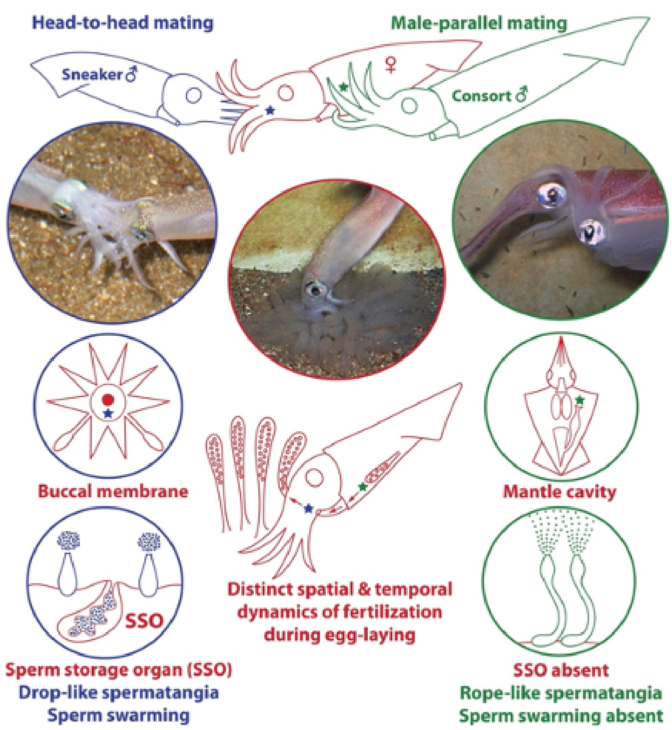
Mating Strategies of Cephalopods

Along the lush, forested coast of northern Japan, in the spectacular Aomori prefecture, masses of spear squid *(Loligo bleekeri)* are gathering. But they're not the only ones: Dr. Yoko Iwata's team have also come to investigate (Iwata, Sakurai and Shaw, 2014). Half a decade earlier, however, Iwata and her colleagues found themselves on the opposite shore, in Hokkaido prefecture, trying to unlock the secrets of cephalopod mating in *L. bleekeri* (Iwata, Munehara and Sakurai, 2005). She decided to bring two males and one female to the laboratories at Hokkaido University— and try her hand as a matchmaker. Fascinated by the complex array of mating tactics that cephalopods employ, both prior to and following fertilisation, Iwata soon returned to Aomori. This time, she intended to uncover the physiological mechanisms underpinning male mating behaviour and female anatomy. But, as she would discover, there was more to it than that. For cephalopods mating is— quite literally — an ART.

Alternate Reproductive Tactics: ARTs

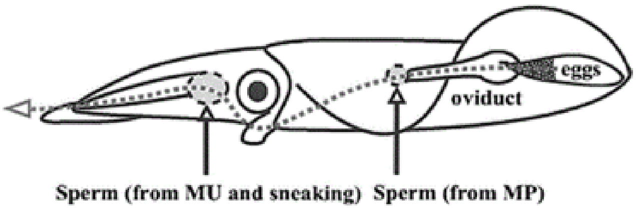
Squid (Teuthoidea) are known to exhibit both inter-and-intrasexual dimorphism. Sound complicated? It isn't.

Their morphologies, physical shape and size, differ; not only between sexes, but within them. Essentially, males come in two sizes: large 'consorts' and small 'sneakers.' In 2019, Dr. José Marian and colleagues (including Iwata) undertook a meta-analysis that examined Alternative Reproductive Tactics (ARTs) in cephalopods.



Mating strategies in cephalopods (Apostólico and Marian, 2019).

Dr. Marian and colleagues distilled these into two principal strategies: 'head-to-head' and 'male-parallel.' As Iwata found in 2005, large consort males place themselves alongside females in the 'male-parallel' position, offering them direct access to the female's mantle cavity, where ova are stored— an ideal locus for sperm deposition. Much to the sneakers' chagrin, consorts also act as bodyguards. So, true to their name, they are forced into subterfuge. Rather than going head-to-head with consorts in physical competition (as the other consorts do), they go 'head-to-head' with females instead. And believe it or not, it works! But how is that possible, when the oviduct is nowhere near the mouth? This is where Iwata's research from 2014 resurfaces.



Sperm-storage sites in *L. bleekeri*. (Wada *et al.*, 2005)

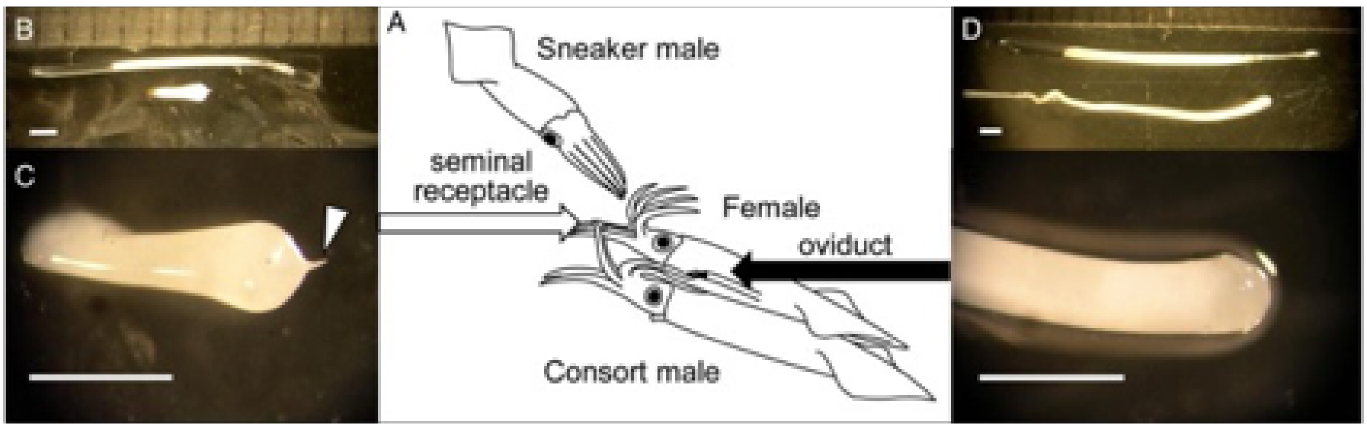
A Tale of Two...Gametes

Back on the Aomori coast, Iwata's team spent two days catching squid— 620 individuals, to be exact— and began measuring them. Of the 328 males, 10 were taken at random, and examined (Iwata, Sakurai and Shaw, 2014). As Iwata discovered during another study, in 2007, female *L. bleekeri* have two special storage sites for the sperm packages called spermatophores: 1) the buccal membrane, near the mouth and 2) the mantle cavity, on the torso (Yoko Iwata & Sakuari, 2007). Presently, however, Iwata was interested in male squid. After opening them up and dissecting the Needham's sac, a tract where sperm are stored, the researchers made a bizarre and novel discovery: there were two different types of sperm...in the same species!

Sneaker males produced small, teardrop-shaped sperm, while consorts made longer and more rope-like sperm. But it begs the question, why do females allow multiple matings in the first place? Isn't that just— promiscuous?

The Profits of Promiscuity

Let's face it: cephalopods are promiscuous. But do they profit? This is exactly what Dr. Zoe Squires was pondering. So, she decided to go SCUBA diving at Port Phillip Bay, Australia— and take a few dumpling squid home with her. Squires knew that polyandry, when females take two or more mates, added genetic diversity and therefore improved overall fitness. However, no one had ever demonstrated it in cephalopods. So, she took up the challenge. What Squires found was eye-opening. At the Victorian Marine Science Consortium near Melbourne, Squires began her experiment: she mated female dumpling squid (*Euprymna tasmanica)* in three ways: 1) single mating event (monandrous 1), two mating events with the same male (monandrous 2), 3) multiple mating events, with multiple males (polyandrous).



Two types of sperm in *L. bleekeri* (Iwata, Sakurai and Shaw, 2014).

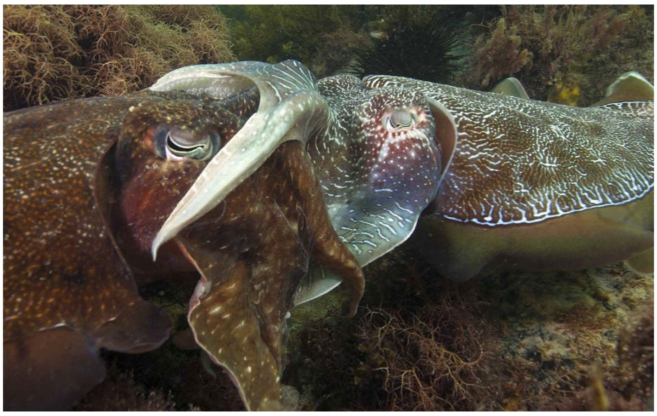
Although monandrous females initially produced larger and more abundant eggs (i.e. more 'egg mass'), it didn't translate into more offspring (i.e. 'hatchling mass'). Meanwhile, polyandrous females produced larger hatchlings — and faster. With proven faster reproduction time and larger hatchings, better equipped to escape predation and capture prey, Squires and her team had the evidence they needed. The profits of promiscuity were, at last, laid bare (Squires *et al.*, 2015; Marshall, Bolton and Keough, 2003). However, Squires was still curious. With multiple paternity confirmed, she wanted to know which gained precedence. In 2005, Iwata had been unable to make the call in *L. bleekeri*. Would Squires have better luck?

Dumpling squid mating (Franklin, Squires and Stuart-Fox, 2012)

In 2017, Squires conducted an experiment on *E. tasmanica* to find out more about 'sperm competition,' which is when sperm from multiple males contend to fertilise a given set of ova (Squires *et al.*, 2015). In cephalopods, it is not uncommon for 4-5 males to gain access to a single female (Shaw and Sauer, 2004), but which male came out on top was still a mystery. After assiduous DNA fingerprinting using five specific gene sequences, called microsatellite loci, Squires discovered that the last male to mate enjoyed a striking 75% success in fertilisation, while the second male dipped to just 54%. Scientists call this, 'last male sperm precedence;' and it makes sense. There is only so much space in the mantle cavity, and the last man standing takes the gold.

In Their Skin: Transient Sexual Mimicry

Meanwhile, a thousand kilometres northwest, another congregation of cephalopods is brewing: giant cuttlefish. At Spencer Gulf, South Australia, masses of giant cuttlefish *(Sepia apama)* have come to mate and spawn. Indeed, nowhere else in the world is such a gathering seen (Naud *et al.*, 2004). Like their squid cousins, cuttlefish (Sepioidea) exhibit male dimorphism, which leaves smaller sneakers at a physical disadvantage. However, they have another trick up their sleeve— or rather *in their skin*. In an extraordinary feat of sexual selection, sneaker males take an enormous risk: they come between consorts and their mates and perform a curious trick: the hopeful sneaker impersonates the opposite sex. Hanlon calls it 'transient sexual mimicry:' sneaker males rapidly alter their skin to produce to the mottled colouration of the female. To complete the guise, they fold their front tentacles in, to mimic the posture of fertilised females.



Sneaker male cuttlefish (right) in 'head-to-head' mating position (photo by Julian Finn)

Amazingly, this behaviour is not only widespread, but also successful (Hanlon *et al.*, 2005). Indeed, Hanlon found that sneaker males succeeded in fooling consorts more than 48% of the time! Utilising genetic fingerprinting Hanlon then proved, for the first time ever, that sexual mimicry could result in immediate fertilisation. So, sneakers may not so sneaky after all— perhaps, they are merely skilled practitioners of an altogether different art.

References

Apostólico, L.H. and Amoroso RodriguezMarian, J.E. (2020). Cephalopod mating systems as models for the study of sexual selection and alternative reproductive tactics: a review. *Bulletin of Marine Science*, 96(2), pp.375–398. doi:https://doi.org/10.5343/bms.2019.0045.

Apostólico, L.H. and Marian, J.E.A.R. (2019). Behavior of 'Intermediate' Males of the Dimorphic Squid Doryteuthis pleii Supports an Ontogenetic Expression of Alternative Phenotypes. *Frontiers in Physiology*, 10. doi:https://doi.org/10.3389/fphys.2019.01180.

Franklin, A.M., Squires, Z.E. and Stuart-Fox, D. (2012). The energetic cost of mating in a promiscuous cephalopod. *Biology Letters*, 8(5), pp.754–756. doi:https://doi.org/10.1098/rsbl.2012.0556.

Hanlon, R.T., Naud, M.-J., Shaw, P.W. and Havenhand, J.N. (2005). Transient sexual mimicry leads to fertilization. *Nature*, 433(7023), pp.212–212. doi:https://doi.org/10.1038/433212a.

Ibáñez, C.M., Pérez-Álvarez, J., Catalán, J., Carrasco, S.A., Pardo-Gandarillas, M.C. and Rezende, E.L. (2019). Sexual Selection and the Evolution of Male Reproductive Traits in Benthic Octopuses. *Frontiers in Physiology*, 10(10). doi:https://doi.org/10.3389/fphys.2019.01238.

Iwata, Y., Munehara, H. and Sakurai, Y. (2005). Dependance of paternity rates on alternative reproductive behaviors in the squid Loligo bleekeri. *Marine Ecology Progress Series*, 298, pp.219–228. doi:https://doi.org/10.3354/meps298219.

Iwata, Y. and Sakurai, Y. (2007). Threshold dimorphism in ejaculate characteristics in the squid Loligo bleekeri. *Marine Ecology Progress Series*, 345, pp.141–146. doi:https://doi.org/10.3354/meps06971.

Iwata, Y., Sakurai, Y. and Shaw, P. (2014). Dimorphic sperm-transfer strategies and alternative mating tactics in loliginid squid. *Journal of Molluscan Studies*, 81(1), pp.147–151. doi:https://doi.org/10.1093/mollus/eyu072.

K., H. and R., H. (2002). Principal features of the mating system of a large spawning aggregation of the giant Australian cuttlefish Sepia apama (Mollusca: Cephalopoda). *Marine Biology*, 140(3), pp.533–545. doi:https://doi.org/10.1007/s00227-001-0718-0.

Marian, J.E.A.R., Apostólico, L.H., Chiao, C.-C., Hanlon, R.T., Hirohashi, N., Iwata, Y., Mather, J., Sato, N. and Shaw, P.W. (2019). Male Alternative Reproductive Tactics and Associated Evolution of Anatomical Characteristics in Loliginid Squid. *Frontiers in Physiology*, 10(10). doi:https://doi.org/10.3389/fphys.2019.01281.

Marshall, D.J., Bolton, T.F. and Keough, M.J. (2003). OFFSPRING SIZE AFFECTS THE POST-METAMORPHIC PERFORMANCE OF A COLONIAL MARINE INVERTEBRATE. *Ecology*, 84(12), pp.3131–3137. doi:https://doi.org/10.1890/02-0311.

Morse, P. and Huffard, C.L. (2019). Tactical Tentacles: New Insights on the Processes of Sexual Selection Among the Cephalopoda. *Frontiers in Physiology*, [online] 10(10). doi:https://doi.org/10.3389/fphys.2019.01035.

Naud, M.-J., Hanlon, R.T., Hall, K.C., Shaw, P.W. and Havenhand, J.N. (2004). Behavioural and genetic assessment of reproductive success in a spawning aggregation of the Australian giant cuttlefish, Sepia apama. *Animal Behaviour*, 67(6), pp.1043–1050. doi:https://doi.org/10.1016/j.anbehav.2003.10.005.

Shaw, P. and Sauer, W. (2004). Multiple paternity and complex fertilisation dynamics in the squid Loligo vulgaris reynaudii. *Marine Ecology Progress Series*, 270(270), pp.173–179. doi:https://doi.org/10.3354/meps270173.

Squires, Z.E., Wong, B.B.M., Norman, M.D. and Stuart-Fox, D. (2012). Multiple Fitness Benefits of Polyandry in a Cephalopod. *PLoS ONE*, 7(5), p.e37074. doi:https://doi.org/10.1371/journal.pone.0037074.

Squires, Z.E., Wong, B.B.M., Norman, M.D. and Stuart-Fox, D. (2015). Last male sperm precedence in a polygamous squid. *Biological Journal of the Linnean Society*, 116(2), pp.277–287. doi:https://doi.org/10.1111/bij.12590.

Wada, T., Takegaki, T., Mori, T. and Natsukari, Y. (2005). Alternative Male Mating Behaviors Dependent on Relative Body Size in Captive Oval Squid Sepioteuthis lessoniana (Cephalopoda, Loliginidae). *Zoological Science*, 22(6), pp.645–651. doi:https://doi.org/10.2108/zsj.22.645.