The Secret Sex Lives of Rotifers

Sex, Asex and Cannibalism

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In just about any wet habitat you can think of – moist forest floor, rain-filled tree holes, ephemeral puddles, village ponds, montane lakes, flowing rivers, sandy beaches, or even municipal sewage effluents, you can be sure to find microscopic creatures called rotifers (*Box* 1, *Figure* 1). These tiny (50-1500 μm) multicellular animals have fascinated naturalists ever since Anton van Leeuwenhoek observed them in a water sample under the simple microscope that he had fabricated, and described their delicate beauty. There are more than 2000 described species of rotifers in the world, and nearly 350 recorded from India.

The successful colonization of all conceivable wet habitats by rotifers is in part due to their highly adaptive reproductive strategies. Of the three orders of the Phylum Rotifera, the rare Seisonidea always reproduce sexually, while in Bdelloidea parthenogenesis is the exclusive mode of reproduction (*Box* 2). Monogononta show both asexual (parthenogenetic) and sexual modes of reproduction.

Monogononts, the largest group of rotifers, show cyclical parthenogenesis, a reproductive pattern found in cladocerans (eg. *Daphnia*) and some insects (e.g., aphids) also. These rotifers reproduce predominantly by parthenogenesis, but switch to sexual reproduction occasionally. The diploid or amictic (2*n*) female produces diploid eggs (called amictic eggs), which develop into diploid females (*Figure* 2). The female may continue this cycle for a greater part of its life and, under optimal conditions in the laboratory, may not resort to sexual reproduction at all. But in nature, in response to some environmental stimulus, the asexually reproducing amictic females switch to sexual reproduction. These environmental stimuli, which initiate sexual reproduction in the population, seem to vary from one species to

Box 1. Rotifers – the 'Wheel Bearers'

Rotifers are among the smallest multicellular (metazoan) animals, some species being no larger than the single-celled ciliate protozoans. The smallest rotifers are ~50 μm and the largest < 1,500μm. Nearly 80% of the recorded species of rotifers belong to the Class Monogononta (possessing a single ovary). Of the other two Classes (possessing paired ovaries), Bdelloidea is represented by >350 species whereas Seisonidea has only two species that live epizoically on the gills of a marine crustacean *Nebalia*.

A typical rotifer has a ciliated region called corona at its head-end. The corona is formed by two concentric discs (*Figure* 1), and the metachronal beating of the cilia arranged on these discs create the impression of a constantly rotating wheel, and hence the name of the Phylum, Rotifera (which means 'wheel-bearing'). This ciliary motion creates water currents, which help the animal not only in locomotion but also in gathering food. The largest rotifers (such as the predatory rotifer *Asplanchna*) define the body size limit beyond which cilia alone are ineffective for locomotion. In a process called suspension feeding (the most common mode in this group of animals), the animal filters out the food particles (algae, bacteria, detritus) present in the parcel of water brought into the buccal region (mouth cavity) of the corona by the cilia. However, many predatory rotifers are also raptorial (i.e. they capture prey).

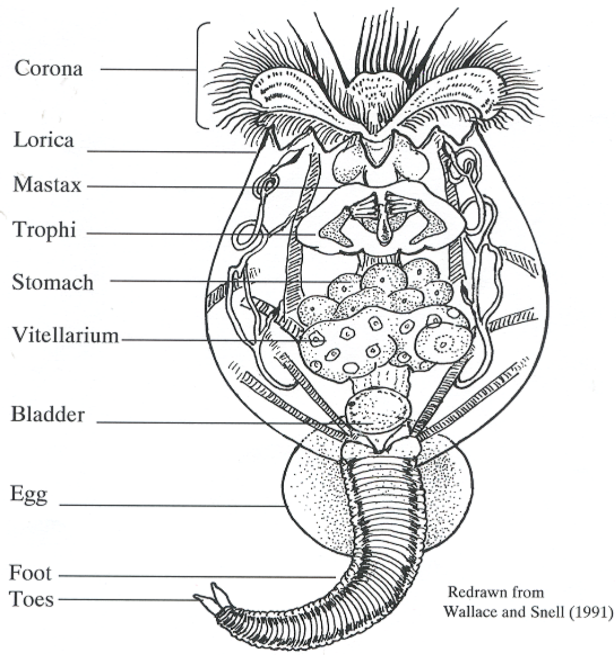

Figure 1. A typical rotifer.

The second characteristic feature of rotifers is a muscular pharynx called mastax, within which are located hard jaws called trophi. Depending on the food habits of the animal, the trophi are variously modified for filtering, grasping, tearing, piercing and sucking food. Because of their complex structure, the trophi are also of taxonomic importance, and are used for identifying different classes, orders and families of Rotifera.

another, and their mechanisms are not yet fully understood. High population densities have been implicated as a stimulus for switching to sexual reproduction in *Brachionus.* In our labo-

Box 2. Bdelloid Rotifers – An 'Evolutionary Scandal'

Sexual reproduction is generally believed to have evolved as a mechanism for producing variation through genetic recombination, the raw material for natural selection to act upon (although more recent theories suggest that it may have evolved primarily to eliminate harmful mutations or as a means to better combat pathogens by increasing the genetic variability of one's offspring). Bdelloid rotifers have been living successfully for millions of years without sexual reproduction and the accruing benefit of genetic recombination, which prompted the noted evolutionary biologist John Maynard Smith to call this group an 'evolutionary scandal'. Recent *in situ* hybridization studies revealed that bdelloid rotifers lack homologous chromosome pairs, a feature characteristic of diploid organisms that undergo meiosis regularly. This suggests that this may not be a case of sexual reproduction being lost secondarily during evolution, but that it probably never existed in these rotifers. A group of molecular biologists at Harvard University are probing the bdelloid DNA to find out if there is some yet-unknown mechanism by which genetic recombination could be taking place in these fascinating creatures.

ratory cultures of *Brachionus*, we generally start finding males and subsequently resting eggs, when the population densities reach very high levels. Photoperiod is known to act as a mictic stimulus in *Notommata*, while in *Asplanchna* a change in the dietary α-tocopherol (vitamin E) appeared to be enough to cause a switch from amictic to mictic reproduction. In many species the switch could occur in < 24 hours.

The amictic females initiate sexual reproduction by producing mictic (sexual) daughters. The duration of mictic daughter production appears to depend on the strength of the mictic stimulus. The mictic females produce via meiosis, haploid (n) eggs, generally smaller than amictic eggs. Only in a handful of species is the same female (called 'amphoteric') able to produce both male and female offspring. The fate of the haploid egg produced by the mictic female is that it will develop into a haploid male if unfertilized, or into a diploid ($2n$) resting egg if fertilized by another haploid male. Thus, the periodic bouts of sexual reproduction in monogonont rotifers can be said to be adaptive in the sense that they lead to the production of resistant resting eggs, which ensure the survival of offspring despite adverse conditions at that time.

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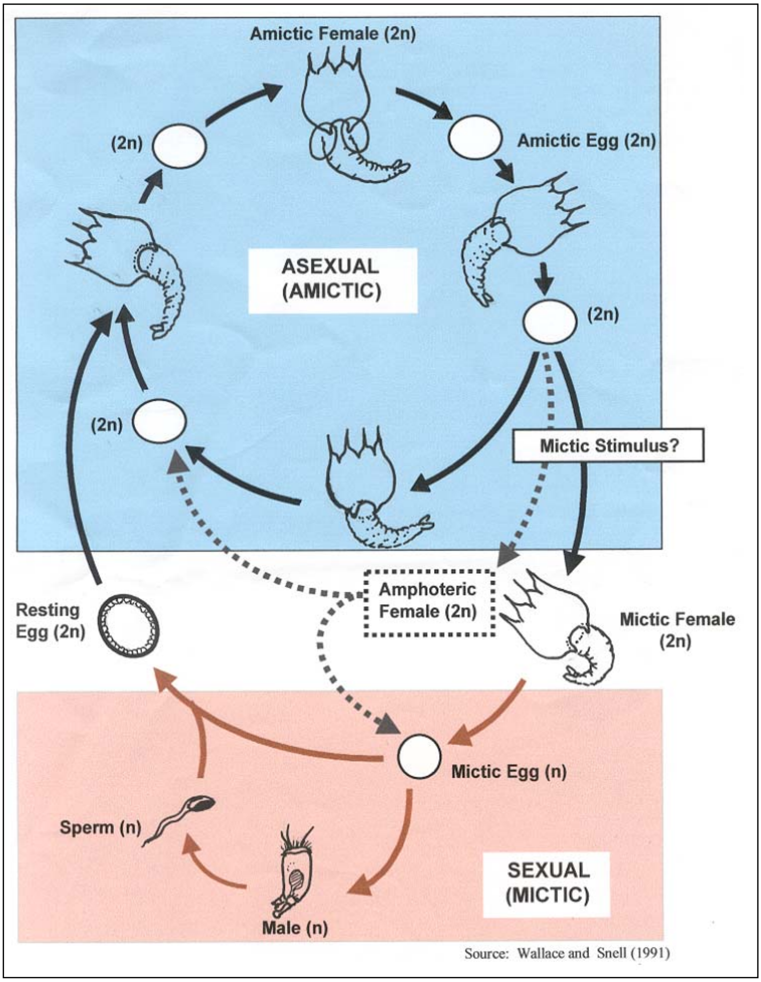
Figure 2. Rotifer life cycle.



The males of many rotifer species are considerably smaller than the females, and their longevity is less than half that of females. Unlike the gracefully moving or, as in some species, sedentary females, they swim fast and constantly, as if in a frenzy, to increase their chances of encountering a conspecific female. Since their only mission in life is to mate with a female and fertilize its eggs, they do not feed, nor do they have any digestive system. A testis and a penis are generally the only prominent parts of their anatomy.

Asplanchna is a predatory rotifer which eats other rotifers, including its own species, if it could grab one. This cannibalistic female doesn't even spare the male who must approach her with extreme caution to perform his duty – copulate and transfer the sperms into the body of the female for fertilizing the eggs. The male, which is about 20-30% smaller than the female, has evolved protuberances on its body wall to reduce the risk of being swallowed by his mate. Upon contact with a potentially predatory female, these protuberances puff up instantly, making the effective size of the male substantially larger, which makes it very difficult for the hungry female to capture.

How does a male rotifer locate its potential mate? Often there are females of more than one species moving around in the medium immediately surrounding it and, therefore, it is not an easy task for the male to locate the right (conspecific) female. Since the male and the female move randomly in the water, their encounters with each other are also random. The female plays no active role in this game and lets the male locate her. The female however secretes a sex pheromone, which the male's chemoreceptors detect (*Box* 3). This chemical substance is not released into the water but bound to the body surface of the female, with the highest concentrations on the corona. The male rotifer

Suggested Reading

[1] J J Gilbert, In *Rotifera*, pp. 231-263, K G Adiyodi and R Adiyodi (eds.), *Reproductive Biology of Invertebrates*, Vol.VI, Pt.A. Oxford and IBH Publ., New Delhi, 1993.

[2] T W Snell, Chemical ecology of rotifers, *Hydro-biologia*, 387/388, 267-276, 1998.

[3] R L Wallace and T W Snell, Rotifera, pp.187-248, In J H Thorp and A P Covich (eds.), *Ecology and Classification of North American Freshwater Invertebrates*, Academic Press, San Diego, USA, 1991.

Box 3. Sex Pheromone of Rotifers

Terry Snell and his co-workers identified the rotifer mate recognition pheromone (MRP) secreted by the female as a 29 kilo dalton glycoprotein (gp29), for which the male has specific receptors. Interestingly, similar chemicals are known to play a role in mate recognition in some other invertebrates also. The rotifer MRP is distributed all over the body surface of the female, but most concentrated in its coronal region. In female *Brachionus plicatilis* exposed to a polyclonal antibody (anti-gp29) developed against this MRP, the probability of a male encounter resulting in mating declines by nearly 80%. Similarly, if a newborn male is exposed to purified MRP, the probability of its mating with a subsequently encountered female is reduced by almost 90%. Even sepharose beads bound with purified MRP were found to elicit mating behaviour in nearly 40% of the males that make physical contact with them. The MRP-receptor in the male is suggested to be glycoprotein-binding protein with lectin-like properties.

The gp29 serves as the species recognition molecule for the male. The molecule is glycosylated with oligosaccharides composed of mannose, glucose, N-acetylglucasamine, fucose and N-acetylgalactosamine. Minor structural changes in the oligosaccharides are enough to produce many species-specific gp29-MRP's and maintain reproductive isolation in nature. Recently, using the MRP specificity as a tool, Snell and co-workers discovered the existence of a sibling species complex in *Brachionus plicatilis*.

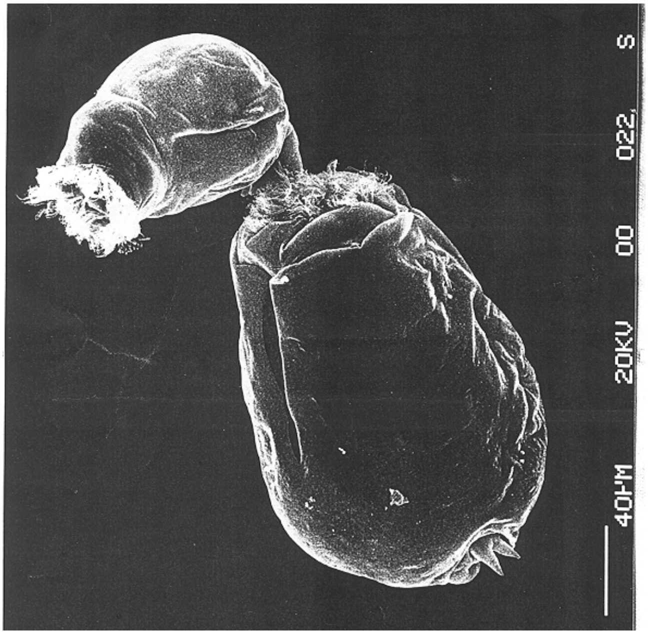


Figure 3. SEM photograph of mating in Brachionus plicatilis.
(Courtesy: Terry Snell)

therefore is not attracted to the female from a distance, but recognizes the conspecific mate only after physical contact with it in one of its random encounters. It has been demonstrated that the male corona has chemoreceptors that specifically bind to the female pheromone.

Once the male recognizes the potential mate as a conspecific female, it swims around the female in circles while maintaining contact with it, a primitive form of courtship behaviour. Then the male attaches its penis to the coronal region of the female (*Figure* 3) and transfers sperm into the body cavity. The entire act lasts < 2 minutes, and in its short life span the male has enough sperm and time to inseminate more than one female.

The fertilized eggs (diploid) become what are called diapausing or 'resting eggs'. These eggs, protected by a thick shell, have the amazing ability to stay viable for very long periods (*Box* 4). Since the natural habitats of rotifers – ponds, pools and puddles, may often completely dry up, resting eggs are the rotifer's way of ensuring survival of its offspring. When the water bodies fill up again during the next rainy season, these resting eggs hatch into diploid females, to start a new generation. On our campus, following an unseasonal heavy rain, a small depression used to fill up to form an ephemeral puddle, which would then dry completely in less than a week. In that short period, one or two species of rotifers would come up seemingly from nowhere and complete their life cycle. Obviously, resting eggs from an earlier season were there. This is an 'egg bank strategy' that helps rotifers to exploit opportunistically a favourable situation when it develops with little or no advance notice. Bottom sediments of many lakes and ponds often have enormous numbers (up to 3000

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Box 4. Embryonic Diapause and Anhydrobiosis in Rotifers

The rotifer resting egg represents an embryonic diapause. Although the ability to produce them is presumed to be an adaptive strategy against adverse environmental conditions, many species appear to produce resting eggs at frequent intervals without any deterioration in ambient conditions. The resting eggs, produced when population densities are very high, settle down into the bottom sediments and lie dormant. They are generally resistant to considerable temperature extremes and, depending on the species, can remain viable for a few months to >30 years.

Although most resting eggs result from sexual reproduction, the production of diapausing asexual (amictic) eggs by *Synchaeta pectinata* has also been reported recently. These eggs were apparently produced in response to starvation caused by low food conditions in the medium. It is not yet known how common asexual egg diapause is in nature.

Bdelloid rotifers also live in habitats that may periodically dry up, but they do not produce resting eggs like monogononts. They have, however, the ability to enter a dormant state called 'anhydrobiosis' (a complex process involving the loss of water accompanied by many physiological and biochemical changes). Anhydrobiotic bdelloids may remain viable upto two decades but return to normal life within minutes or hours.

The hardy resting eggs of rotifers are probably one of the mechanisms by which many species of rotifers may have managed to achieve a cosmopolitan distribution. For instance, enough resting eggs from a far-off temperate area could attach themselves to the feet of migratory aquatic birds and hitchhike to a tropical wetland and eventually establish the species there.

Rotifer resting eggs have also proved to be a big advantage in aquaculture. Because of their small size, rotifers (particularly the species, *Brachionus plicatilis*) are the most widely used first food for rearing fish and shrimp larvae in aquaculture. Resting eggs of *Brachionus*, now commercially available, can be stored for long periods and hatched out when needed for a starter culture, without having to depend on nature.

per square centimeter have been recorded) of resting eggs of different species of rotifers deposited over many years, each patiently waiting for some specific environmental cue to hatch. Not much is known as yet about the nature of such environmental cues. The sediments in which the eggs rest are generally cold, anoxic and dark, and their displacement from the sediments to the illuminated, warmer and well-oxygenated water above might perhaps serve as a stimulus. Seasonal limnological events such as 'spring-overturn' may also facilitate such displacement of resting eggs. Limited observations available suggest that in nature rotifer resting eggs hatch sporadically, a few at a time, at frequent but irregular intervals.

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