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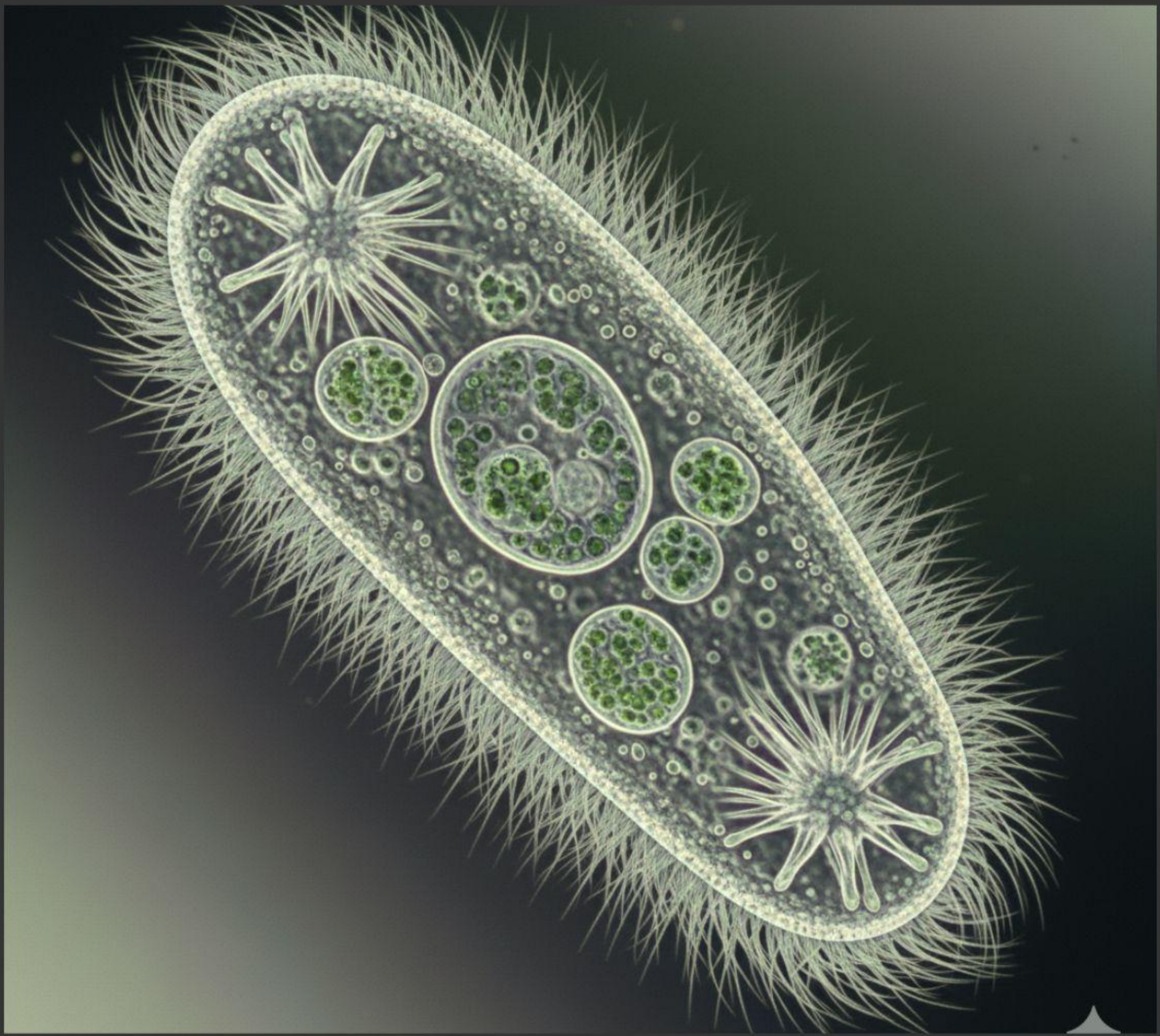
Chapter 2

INVERTEBRATES CHAPTER SERIES

Paramecium caudatum



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Paramecium caudatum

(The slipper animalcule)

CLASSIFICATION
(SYSTEMATIC POSITION)
Phylum: Protozoa
Subphylum: Ciliophora
Class: Ciliata
Subclass: Holotricha
Order: Hymenostomatida
Suborder: Peniculina
Genus: *Paramecium*
Species: *caudatum*

INTRODUCTION

Paramecium belongs to the class Ciliata. These animals possess the characteristic cilia for locomotion. The common species P. *caudatum* has longer cilia at the extreme posterior end of the body (caudal end), forming a caudal tuft. Hence, the species got its name, caudatum. *Paramecium* has two types of nuclei: a large bean-shaped macronucleus controlling metabolic activities and a small round micronucleus which controls the reproductive processes. This phenomenon of having two morphologically different nuclei is termed nuclear dimorphism. It also possesses a unique method of sexual reproduction called conjugation. *Paramecium* has about 10 known species differing in shape, size and structure. The largest species is P. *caudatum*. It occurs widely and abundantly. P. *aurelia* has one macronucleus and two micronuclei, while P. *multimicronucleatum* has many micronuclei. P. *bursaria* is another species which is green due to the presence of the symbiotic alga, *Zoochlorella* in its body. P. *caudatum* Is commonly known as a slipper animal. It is one of the most common species of *Paramecium* having a worldwide distribution. It is found in freshwater ponds, pools, ditches, streams, rivers, lakes, reservoirs, etc. It is usually abundant in those waters which contain a good amount of decaying organic matter. It is commonly found in ponds or slowly running water streams having organic matter or plant vegetation. Paramecia can be seen in a drop of water in which they live with a simple compound microscope.

EXTERNAL STRUCTURE
(MORPHOLOGY)

1. Size and shape. As the name, slipper animal suggests, *Paramecium* is a spindle-shaped microscopic organism **(Figure 1)**. Pellicle is the layer of *Paramecium* below the plasma membrane which is responsible for the maintenance of its definite shape. If sizes are taken into consideration, its species vary in length from 80µ to 350µ. P. *caudatum*, the largest species, measures between 170µ and 290µ. P. *aurelia* is about 120 to 250µ long. The individuals of the same species may exhibit morphological and physiological differences. The body of the *Paramecium* has a dorsal and a ventral surface. Ventral surface is also called the oral surface (having an oral groove) and the dorsal surface is termed as the aboral surface. Because of its slipper-like shape, the *Paramecium* is sometimes called the slipper animalcule. It has also been named 'chausson' by Joblot due to its slipper shape. The body is blunt and rounded at the anterior end and pointed at the posterior end with a bunch of elongated cilia, the caudal tuft. Ventral or oral surface has a prominent depression, called the oral groove. This is the first part of the oral apparatus. The oral groove leads into a vestibule which in turn leads into the buccal cavity having a mouth (cytostome). Thus, the mouth is not on the outer surface, in fact it is located deeper within its body.

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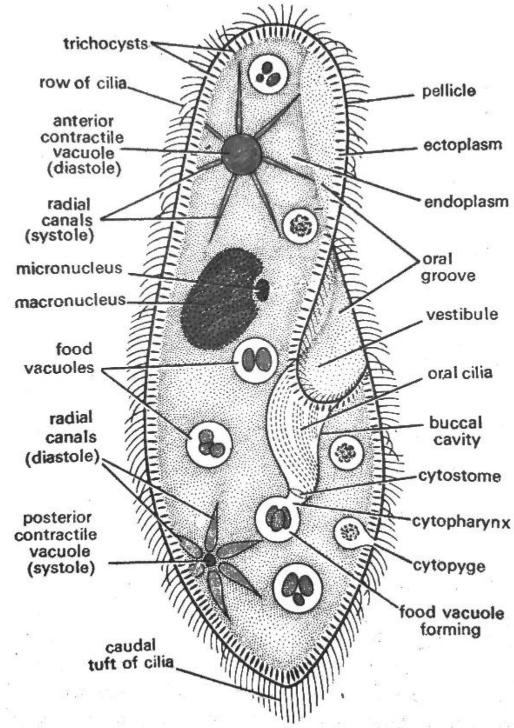

Figure 1. Ultrastructure of *Paramecium caudatum*

2. Pellicle (Outer envelope). It is a living, clear and elastic outer membrane of *Paramecium*. When stained specimens are observed under light microscope, the pellicle appears to be a regular series of hexagonal depressions. A single cilium emerges from the middle of each hexagon **(Figure 2)**. Further studies have shown that the hexagons have cavities below them which are termed as 'alveoli' (singular is alveolus).

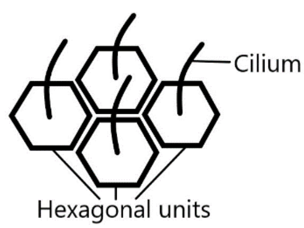

Figure 2. A diagrammatic representation of pellicle showing hexagonal units

Thus, the pellicle is formed of a continuous sheath having two membranes - outer alveolar and inner alveolar membranes. Plasma membrane remains the outermost layer of *Paramecium* as the outer alveolar layer lies in close contact with the cell membrane. Hence, the series of membranes from outer to inner side is (i) Plasma membrane (ii) Outer alveolar layer (iii) Inner alveolar layer. Below the pellicle, a system of fibres is found which is called 'infraciliary system'.

Functions of Pellicle. As pellicle is a firm layer, it provides a definite shape to the organism. Pellicle has an elasticity factor which allows *Paramecium* to pass through narrow passages. It also limits the cytoplasm. The alveoli of the pellicle also give stability to the plasma membrane of the organism. Pellicle is semipermeable, hence it can regulate the exchange of materials.

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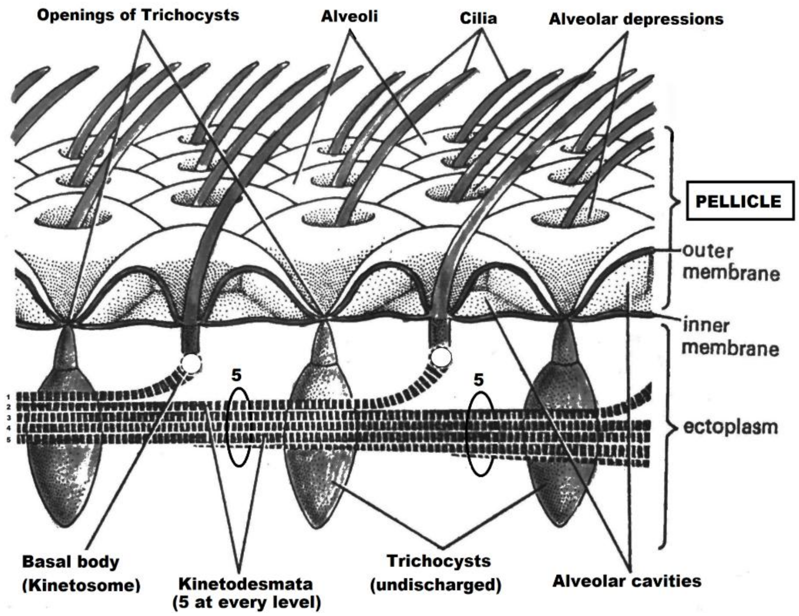

Figure 3. A portion of pellicle in *Paramecium*

3. Cilia. Whole of the body surface of *Paramecium* is covered by numerous fine projections called cilia. A single cilium measures about 10µm in length and 0.25µm in diameter. Hence, cilia are microscopic structures which help the *Paramecium* in locomotion. There can be around 10,000 cilia covering the whole body surface of the animal. As already discussed, cilia are arranged in a regular fashion and are incorporated in the pellicle. Each cilium arises from a basal body and projects out from the centre of each hexagonal unit of the pellicle **(Figure 2)**. The length of cilia is the same throughout, but the cilia present at the posterior end are longer than the rest of the body. These longer cilia form a group at the posterior end known as the caudal tuft. Each cilium has a 9 + 2 arrangement of microtubules.

4. Infraciliary system (System below the pellicle)*.* Below the pellicle, there is located the infraciliary system, sub-ciliary system or Kinety system is located in the ectoplasm which includes basal bodies, kinetodesmata and a motorium. It is also regarded as a primitive nervous system which controls the ciliary beats.

(a) Basal bodies (Kinetosomes). Each cilium bears a round body at its base called 'basal body' or kinetosome. Each cilium has a 9 + 2 arrangement of microtubules which means every cilium has 2 microtubules in the centre and 9 on the periphery. Basal body at the base of cilium lacks the central 2 microtubules and has a 9 + 0 arrangement. Thus, basal bodies or kinetosomes are formed only by the peripheral fibres of cilia which run continuously into it. The basal bodies are self-duplicating structures and are responsible for the origin of new cilia.

(b) Kinetodesmata. If we see below the level of the pellicle, it's the ectoplasmic layer of cytoplasm. Here in this layer, special striated fibrils are present which are associated with the basal bodies. A single fibril (kinetodesma) is found attached to every single basal body or kinetosome. A bundle of kinetodesma is termed as kinetodesmata. If we go by the diagram, below each basal body, runs a bundle of kinetodesmata having 5 kinetodesmal fibres running parallel to the pellicle. Beauty of the arrangement lies in the way they arrange themselves. As a new fibril (kinetodesma) arises from a basal body, one last kinetodesma ends. Thus, in the kinetodesmal bundle, where one fibril lasts, another fibril is added from the basal body. It results in a continuous bundle having a thickness of 5 kinetodesmal fibres. The number of fibrils remains constant (5) in the kinetodesmata. Researchers have found that kinetodesmata coordinate ciliary beat and movement in *Paramecium* but the evidence for the same is not confirmed.

Kinety system. The kinetosomes (basal bodies) along with their kinetodesmata constitute a structural unit known as "Kinety". This kinety system is a characteristic feature of ciliates. The Kinety system has been found to play an

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important role in the morphogenesis of Protozoans. For example, in *Paramecium*, one set of kinety is solely responsible for the development of mouth structure. A new mouth fails to develop if this kinety is removed experimentally. The number of kineties is species-specific. Thus, a particular species of *Paramecium* has a definite number of kineties.

Kinetosome + Kinetodesmata = Kinety System

(c) Motorium. Left dorsal wall of the cytopharynx bears a darkly stained, bilobed structure called 'neuro-motorium'. Generally, the part of the nervous system in an animal that is concerned with movement is known as the motorium. As Paramecium is a unicellular animal, motorium is considered a nerve centre. All the kinety systems converge end up here on the motorium **(Figure 4).**

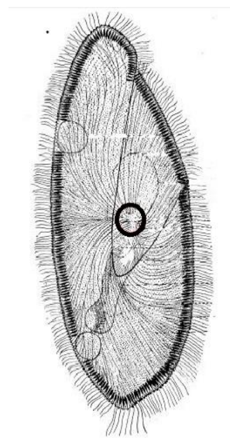

Figure 4. Location of motorium in Paramecium (black circle)

All the kineties (kinetosomes and kinetodesmata) along with the motorium form the infraciliary or the neuromotor system in *Paramecium*.

5. Trichocysts. These are spindle-shaped or oval organelles of about 4 microns length which are present in the ectoplasm and help in protection. The trichocysts are present in the perpendicular direction to the pellicle. They are present alternating with basal bodies. Each undischarged trichocyst consists of an elongated shaft and a terminal pointed tip, called the spike or barb, covered by a cap. The matrix of the shaft consists of a dense mass of a fibrous protein, called trichinin. In the undischarged trichocyst, trichinin fibres remain condensed forming a cross-striated lattice work. Whenever the *Paramecium* senses fear of some predator, trichocysts are discharged. The caps are taken off and the spikes come out as a protective mechanism. Discharge of trichocysts may be triggered by mechanical, chemical or electrical stimulation and takes a few milliseconds. This process involves the unfolding of the lattice of trichinin fibres, thus creating a pressure for the spike to release out. Some researchers believe that trichocysts discharge and anchor the animal to substratum when it feeds.

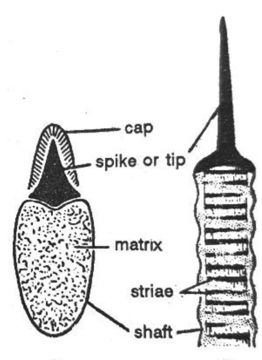

Figure 5. Trichocysts in undischarged and discharged states.

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6. Cytoplasm. Underneath the pellicle, the body of Paramecium contains a fluid-like substance, the cytoplasm. It is clearly differentiated into two regions:

(a) Ectoplasm. Peripheral dense zone of cytoplasm is called the ectoplasm. It contains the structures like infraciliary system having kinetosomes and kinetodesmata; and the trichocysts. All these structures reside in ectoplasm.

(b) Endoplasm. Central, granular and semi-fluid zone of cytoplasm is called the endoplasm. It contains the cell components like macro and micronuclei, mitochondria, contractile vacuoles, golgi bodies, ribosomes, crystals, reserve food granules, etc. In P. *bursaria*, the endoplasm is filled with symbiotic alga *Zoochlorella*.

7. Nucleus. *Paramecium* possesses two types of nuclei which are present in the endoplasm. Thus, *Paramecium* is considered to be heterokaryotic. In P. *caudatum*, there is a large bean shaped macronucleus and a small round micronucleus. In P. *aurelia*, two micronuclei are present along with a single macronucleus. Thus, it has a total of three nuclei. P. *multimicronucleatum* has a number of micronuclei.

(a) *Macronucleus*. It is roughly bean shaped and with an inconspicuous nuclear membrane. It is polyploid and possesses many nucleoli. It contains greater amounts of DNA in it as compared to micronucleus. Macronucleus is the somatic or vegetative nucleus and controls the metabolic activities in *Paramecium*.

(b) *Micronucleus*. It is usually spherical and is present in the depression of the macronucleus. It has a nuclear membrane and is diploid (2N) in nature. P. *caudatum* lacks nucleoli inside the micronucleus. It is responsible for controlling the reproductive activities of *Paramecium*.

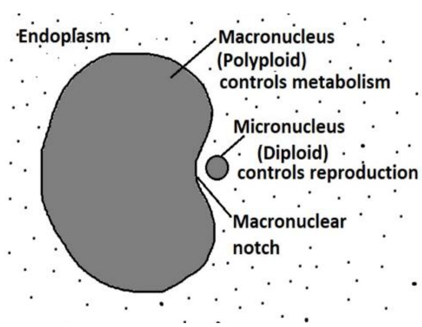

Figure 6. Types of nuclei in *Paramecium caudatum*

8. Contractile apparatus. In *Paramecium*, there exists a fixed number, position and out-pores of the contractile vacuoles. These are meant for osmoregulation. There are two contractile vacuoles, occupying anterior and posterior positions in the endoplasm of the organism. They lie close to the dorsal surface of *Paramecium* and have permanent out-pores on the dorsal sides. Here, in *Paramecium*, the contractile vacuoles have fixed positions. So, they cannot move here and there to collect the extra water for removal as a part of osmoregulation. Thus, each contractile vacuole has 6 to 10 spindle-shaped canals which are known as radial canals. These canals give a star shape to the contractile vacuoles. Radial canals run into various parts of the organism serving to collect water from different sites. Radial canals collect water and fill the central contractile vacuole. Finally, this vacuole contracts and the water is expelled out of the out-pores from the dorsal side. Vacuoles are retained and they start to collect water again. Further studies revealed the presence of endoplasmic reticulum tubules and nephridial tubules along with the radial canals. All these tubules, canals along with the main contractile vacuole forms the contractile apparatus.

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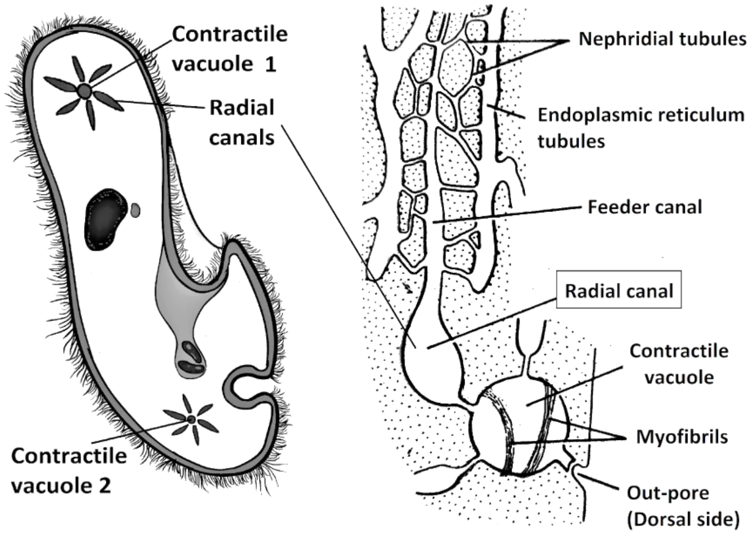

Figure 7. Contractile apparatus in *Paramecium*

9. Food vacuoles. The non-contractile vacuoles formed in the process of ingestion and having food are the food vacuoles. These can be seen moving with cyclosis in the body of *Paramecium*. They differ in shape and size according to the nature of ingested food particles. Generally, they are round in shape. These are also called 'gastrioles' or 'temporary stomachs'. They are also termed phagosomes (solid food) or pinosomes (liquid droplets). Food vacuoles are the centres of intracellular digestion. Further, the lysosomes attach with the food vacuoles to pour their enzymes over the food.

10. Oral apparatus. The oral groove leads ventrally and posteriorly as a tubular structure, called vestibule. Vestibule leads into the buccal cavity. Further the buccal cavity opens into a tube like structure, the cytopharynx through a narrow aperture, the cytostome (mouth). At the end of cytopharynx, the food is poured into a sac-like structure called the food vacuole. Water also enters into the food vacuole alongwith the food.

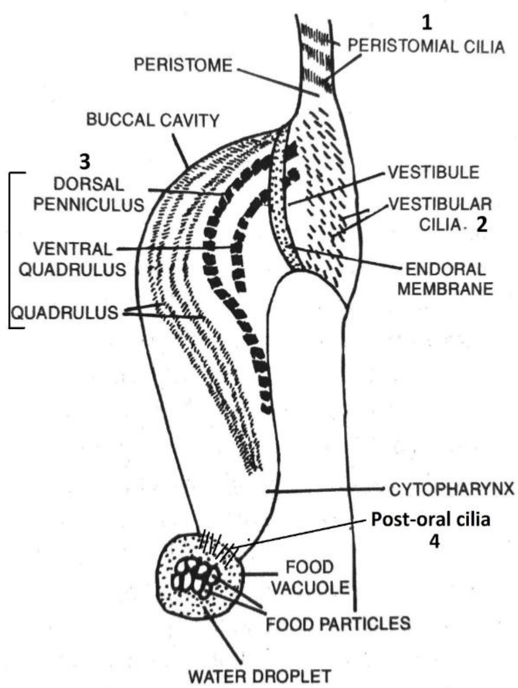

Figure 8. Oral apparatus and ciliature in *Paramecium*

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Buccal ciliature (Cilia of the oral apparatus).

(i) Peristomial cilia: These are the cilia of the peristome or the oral groove. These cilia beat inwardly at an angle to form a water current towards the oral groove which is helpful in feeding.
(ii) Vestibular cilia: These cilia are smaller in size and are arranged in rows. At the junctional point of vestibule and buccal cavity, a row of cilia forms the 'endoral membrane'. This membrane is a filter for the food which allows only the fine food particles to pass into the buccal cavity.
(iii) Buccal cavity: At the left side of buccal cavity are present three groups (Dorsal peniculus, Ventral peniculus and Quadrulus), each having four rows of cilia. These extend upto the posterior end of buccal cavity. These three ciliary groups constitute the 'membranelles'**.** Cilia of these membranelles beat in such a manner that the filtered food particles are passed down the buccal cavity as well as aggregated into a food mass.
(iv) Post-oral cilia: These cilia are longer in length than the normal cilia of the body. These are present in the cytopharynx. The food mass formed by the membranelles is brought forward and put into the food vacuole by these cilia.

11. Cytopyge (Anus). Cytopyge or the cell anus is present near the posterior end of the body of Paramecium, a little behind the cytostome towards the right side. Here at the cytopyge, ectoplasm and pellicle are weak and thin. At the time of egestion, this minute aperture, the cytopyge or cytoproct is visible. This point serves as the out-pore of the organism. The food vacuole with waste materials (residual body) attaches to this point and the contents are released out from cytopyge. As the pellicle and ectoplasm are thin over here, these might break off temporarily and replenish after the removal of the undigested food.

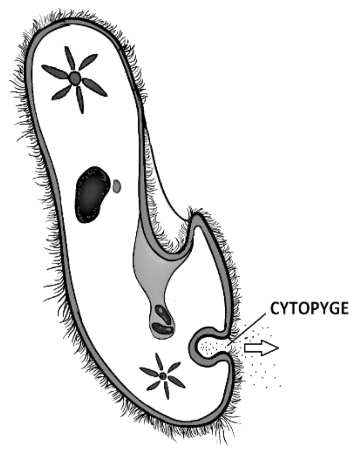

Figure 9. Cytopyge in Paramecium

CULTURE OF PARAMECIUM

Paramecia can easily be grown in wide jars or petri dishes filled with boiled pond water or Chantey's medium and with 10 drops of milk added weekly. The jars are kept away from direct light to allow bacteria to grow. Bacteria serve as food for the multiplying Paramecia. After a few days, Paramecia can be found in the petri dishes. As the Paramecia are found swimming in the water medium, the sample of the water should be taken from the top or centre and put over the microscopic slide. It should be covered with a cover slip before being observed under the light microscope. Paramecia can be seen swimming here and there in an anticlockwise spiral fashion.

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PHYSIOLOGY

1. Locomotion

Locomotion is the change of place for the need of shelter or food. *Paramecium* has a streamlined body which helps it to swim in water with minimum drag (frictional force). As it is a Ciliate, the rapid swimming is facilitated by the beating of fine hair-like cilia that cover the animal's entire body. The speed of *Paramecium* while swimming can be around 1500 micrometre per second. To understand the mode of locomotion in *Paramecium* we have to discuss the beat of a single cilium and their coordination.

a. Ciliary beat. When the Paramecium locomotes, every cilium oscillates like a pendulum. Each single oscillation of a cilium has two phases: a fast effective stroke and a slow recovery stroke. During the fast effective stroke, the cilium becomes rigid and moves backward striking the water. It pushes the water backwards. Thus, the body of *Paramecium* is propelled in the forward or opposite direction. On the other hand, in the recovery stroke, cilium becomes soft and comes back to its original position. Thus, water is pushed back only on the effective stroke. Every recovery stroke is followed by the next effective stroke. for a continuous push to the animal.

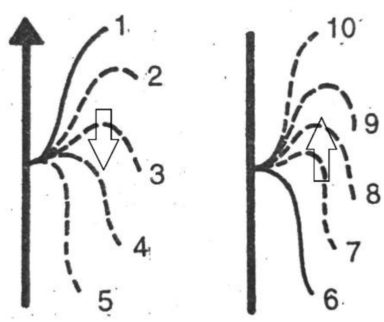

Figure 10. Ciliary beats: Effective (A) and Recovery (B) strokes of a cilium

Metachronal rhythm. In *Paramecium*, all the cilia of the body move in a characteristic wave-like manner, called metachronal rhythm. The cilia in a longitudinal row beat in a wave beginning at the anterior end and progressing backwards. Hence, a cilium of a longitudinal row always moves in advance of the one behind it. All the cilia of a transverse row beat simultaneously. Thus, Paramecium can move due to these metachronal waves.

Cilia moving in groups

Figure 11. Metachronal rhythm shown by groups of cilia

b. Swimming

Forward motion. With the help of metachronal waves of the cilia, Paramecium can swim in the water medium. But, the forward swimming motion is not in a straight path. Infact, it follows an 'anti-clockwise spiral' path. There are two reasons for this kind of movement. (i) The cilia in *Paramecium* do not beat exactly in the backward direction but at some angle. Thus, the cilia move in a somewhat oblique direction pushing the water at an angle to the animal's line of the body. Hence, the animal rotates over to the left on its long line of axis. (ii) The cilia of the oral groove move rapidly so as to turn the anterior end away from the oral side and thus, the animal moves in circles. These two factors cause the movement of the animal rotating about its axis in an anticlockwise direction.

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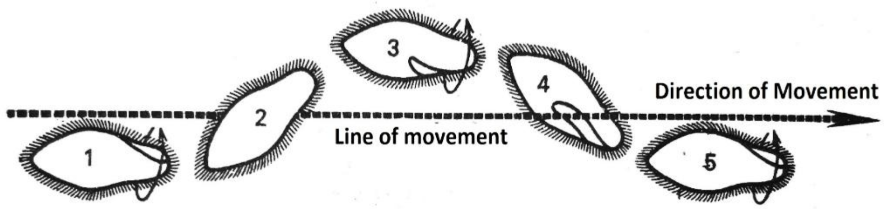

Figure 12. Anti-clockwise spiral forward movement in *Paramecium*

Backward motion. Whenever the *Paramecium* feels some hindrance in the path and has to move backwards, a *Paramecium* follows a straight course rather than anticlockwise or spiral motion. In this case the metachronal wave passes from the anterior end towards the posterior end.

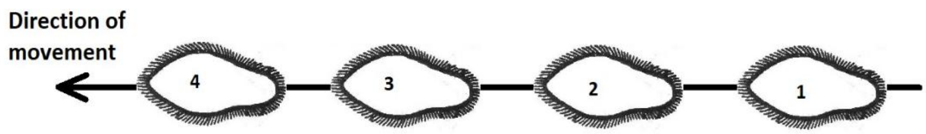

Figure 13. Backward movement in *Paramecium*

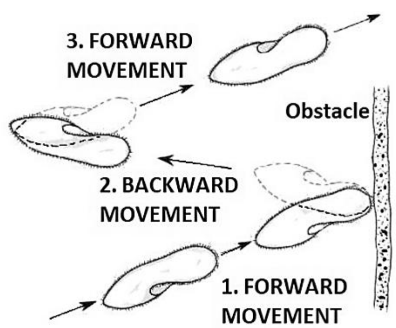

Figure 14. Forward and backward movement in *Paramecium* in the presence of an obstacle

While forward swimming locomotion, whenever a *Paramecium* observes an obstacle in the path, it reacts to it by moving away from it. It can choose to move backwards to avoid it. When it touches the obstacle, it moves backwards in a straight path with its posterior sharp end in the forward direction. When it reaches a safe distance from the obstacle, it continues its normal forward swimming anticlockwise spiral locomotion with its anterior blunt end forwards.

c. Creeping. The *Paramecium* can creep along the substratum also. It is a slow type of locomotion in which the cilia of the ventral side are used to creep over the surface. The animal uses its ventral cilia and simply glides over the debris or particulate matter. The animal can bend and squeeze through narrow spaces or gaps while creeping.

NUTRITION

1. Mode of nutrition. *Paramecium* is a holozoic feeder and feeds on solid food particles. The food may consist of bacteria. It has been observed that about 2 to 5 million of *Bacillus coli* are eaten up by a single Paramecium in a single day. Paramecia also feed upon small Protozoans, unicellular plants, algae, diatoms, yeasts and even small bits or particulates of animals and vegetables. It can reject the larger food particles and can select the desired particulate matter. That is why *Paramecium* is known as a filter feeder. P. *bursaria* is a green coloured species due to the presence of the symbiotic *Zoochlorella* inside its cytoplasm. It can live for long periods on food substances manufactured by *Zoochlorella*. During scarcity of food, it can digest even its own *Zoochlorella* and can live without them.

2. Ingestion. *Paramecium* can sense the presence of food and swims to places where it can procure its food. Food is ingested by the specialized system called the oral apparatus. Ultimately, food reaches the cytostome lying at the bottom of the buccal cavity and is poured into a food vacuole.

Cilia of the oral groove including peristomial cilia beat in a definite way to drive a current of water towards itself. Along with the water, food particles enter the oral groove and move towards the vestibule. Further, the ciliary tracts of vestibule direct the food particles into the next part, buccal cavity. *Paramecium* is known to be a selective feeder as it can select the food to be ingested. Different kinds of food particles may be carried along with water current into the vestibule, but only the selected food of appropriate size passes down the buccal cavity. The path followed by the food while coming inside the vestibule is termed 'selection path'. The bigger or unwanted food particles are rejected and are thrown out of the vestibule by the reverse action of cilia. This filtration process is carried out by the endoral membrane which is present at the joint of vestibule and buccal cavity. The passage followed by the rejected food particles out of the vestibule is termed 'rejection path'.

After the selection of the food particles, cilia of membranelles of buccal cavity beat and drive the selected food particles through the cytopharynx upto the cytostome or mouth. Food gradually collects at the bottom of cytopharynx and is poured into the food vacuoles which is pinched off from the membrane of the cytopharynx. When the food vacuole gets filled with food, it is released into the cytoplasm or endoplasm for further action. Next food vacuole formation depends upon the food supply and rate of feeding. It may take 2 to 5 minutes to make the next food vacuole which is again pinched off into the endoplasm and so on.

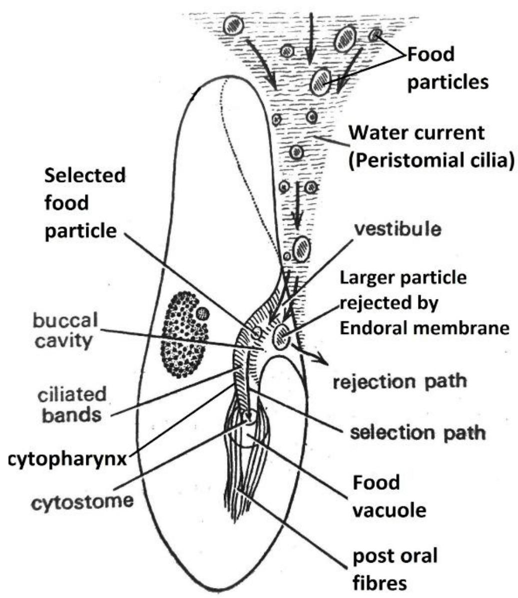

Figure 15. Ingestion of food particles by *Paramecium*

3. Digestion. When the food vacuole is formed and is pinched off into the endoplasm, it moves inside *Paramecium* in more or less definite paths. These paths are achieved by the cytoplasmic streaming movements or 'cyclosis'. As the vacuoles are formed at the level of the cytostome, they are released into the endoplasm. Thus, if *Paramecium* is eating voraciously, many food vacuoles may be formed quickly and can be seen moving inside the endoplasm. The vacuoles move inside the endoplasm with the cyclosis in two different paths. This may be in the shape of digit zero "0" or digit eight "8". In zero type path, first, the vacuoles are taken along the endoplasm to the posterior side and then to the blunt anterior side. After that these are again carried posteriorly reaching the cytopyge at the end. In the case of an eight shaped path, first the food vacuole streams around the cytopharynx, then it is taken to the anterior blunt side. After a cross loop, the vacuoles travel towards the posterior sharp end, finally reaching the cytopyge.

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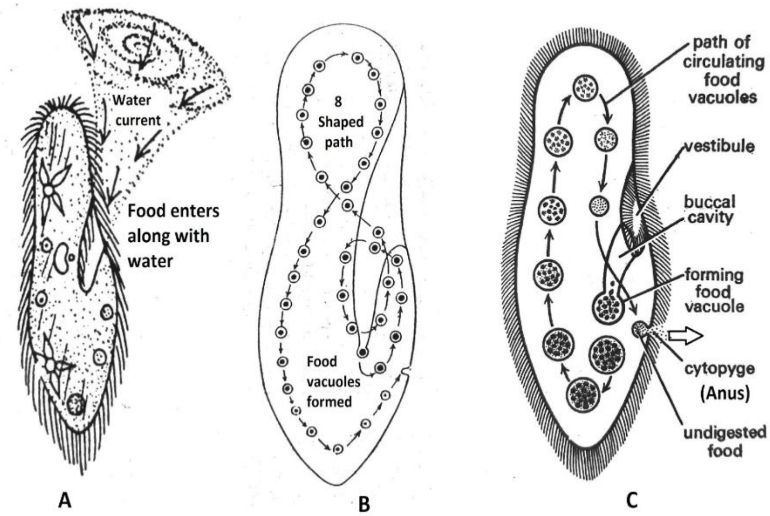

Figure 16. Ingestion of food (A) and paths (B,C) followed by food vacuoles in *Paramecium*

Attachment of lysosomes. As the food vacuoles (phagosomes) move inside the endoplasm, lysosomes attach themselves to the vacuoles forming 'phagolysosomes'. The medium of the food vacuole is kept acidic first to kill any of the food (prey) if needed. Gradually, the medium becomes less acidic or alkaline so as to facilitate the enzymatic digestion by the lysosomal enzymes. Digestive enzymes including carbohydrases, proteases and lipases are poured by the lysosomes into the food vacuoles. Products of digestion include glycogen and fat globules.

4. Absorption. Both digestion and absorption of food takes place during the movement of food vacuoles in the cytoplasm. After the digestion of food inside food vacuoles by the lysosomal enzymes, the final products diffuse out from the food vacuole into the surrounding cytoplasm. This process is known as absorption. The end products are absorbed more and more into the cytoplasm while the food vacuoles keep on streaming inside the endoplasm.

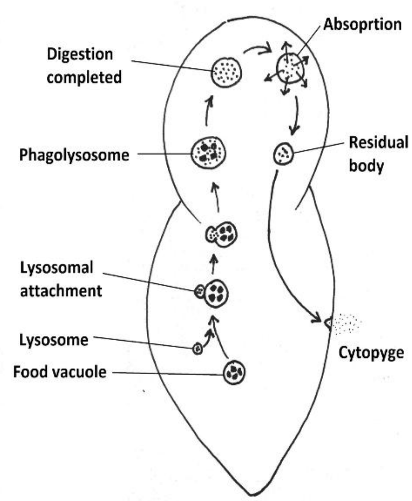

Figure 17. Digestion, absorption and egestion processes

5. Egestion. After the absorption of the final products from the food vacuoles, vacuoles become smaller. These smaller vacuoles are now containing only the waste or undigested materials because all the essential materials have been absorbed. This vacuole having only the waste materials is termed as 'Residual body'. These bodies finally reach the cytopyge and attach to this site. As the pellicle is thin over this spot, the wastes are egested out of cytopyge and the membrane is replenished. Thus, cytopyge is taken equivalent to the anus in *Paramecium*.

RESPIRATION

Oxygen intake. *Paramecium* lives in a freshwater environment which has a high concentration of oxygen as compared to the cytoplasm of the organism. *Paramecium* also uses the available oxygen for the oxidation of food. Hence, the oxygen content inside the cytoplasm of the organism further decreases. This results in the formation of a concentration difference or gradient of oxygen in between the cytoplasm (low) and the outer water medium (high). Due to this concentration gradient, oxygen enters the *Paramecium* from outside water through the semi-permeable pellicle by the process of diffusion. This entered oxygen is further used for the oxidation of food and the cycle goes on.

Carbon dioxide release. When the oxygen has been used by the *Paramecium* for oxidation of food molecules, it results in the formation of carbon dioxide in the cytoplasm. Now, the concentration of CO_2 starts to rise. As the concentration of CO_2 is less in the outside water, it develops a concentration gradient of CO_2 in between cytoplasm and outside water. This condition is exactly the opposite of the condition for oxygen concentration. Ths, CO_2 moves out of the Paramecium through the pellicle by the process of diffusion.

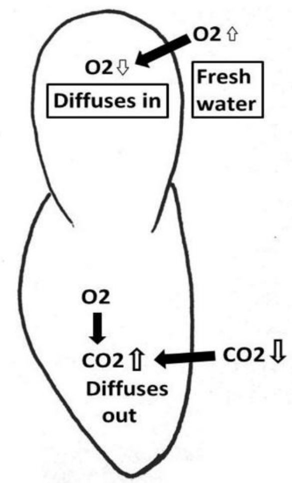

Figure 18. Diffusion of oxygen and carbon dioxide

EXCRETION

Excretion involves the removal of nitrogenous waste products. *Paramecium* excretes out the nitrogenous wastes in the form of ammonia. The food that is eaten up by *Paramecium* contains a good amount of proteins. The catabolic reactions in proteins result in the formation of ammonia. Ammonia is a highly toxic compound and needs to be removed. Ammonia simply diffuses out into external water because its concentration is always higher in the body of *Paramecium* as compared to the outside water. Ammonia reacts with the available water inside the cytoplasm of the organism to form ammonium ions. These ammonium ions actually diffuse out of the body into the outside water simply by diffusion. Crystals present in cytoplasm are also excretory products, which get dissolved and eliminated with the fluid of contractile vacuoles.

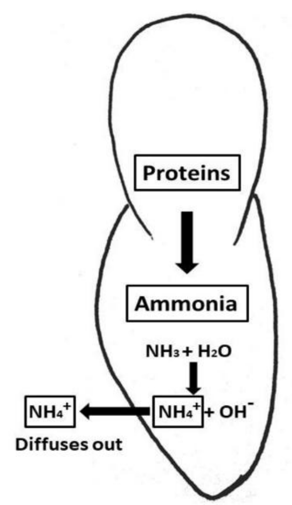

Figure 19. Excretion of ammonia as ammonium ions

OSMOREGULATION

Need of osmoregulation. The regulation of water and salt inside the body of *Paramecium* is known as osmoregulation. As the *Paramecium* lives in freshwater having low salt concentration, there is always a

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concentration gradient in between the cytoplasm of *Paramecium* (high) and the outside water (low). Due to this concentration gradient, the water keeps on coming towards the inside of the organism. This process is called 'endosmosis'. Due to the continuous inflow of water inside the body of *Paramecium,* it swells up. When the food is metabolized, it results in the formation of metabolic water. Water also enters the body of *Paramecium* along with the food particles. Thus, an excessive level of water is observed inside the cytoplasm. This extra water has to be removed so as to keep the body in shape and prevent it from bursting. Hence, the removal of this extra water is known as osmoregulation. This is achieved by the two contractile apparatus present at the anterior and posterior sides.

Mechanism. Contractile apparatus includes the contractile vacuoles, radial canals, nephridial tubules and endoplasmic reticulum tubules. Contractile vacuoles assisted by contractile myofibrils, contract (systole) and relax (diastole) at regular intervals to expel the extra water out. Water from the cytoplasm is secreted into the tubules of endoplasmic reticulum from where it flows into the nephridial tubules. From here it enters the feeder canals to accumulate in radial canals. From the radial canals, the water ultimately reaches the central contractile vacuole. When the vacuole attains a good amount of water, it contracts and discharges the water to the outside through a pore in the pellicle on the dorsal side. Posterior contractile vacuole pulsates faster than anterior vacuole because of the large amount of water being delivered into the posterior region by cytopharynx. Thus, both the contractile vacuole work simultaneously to remove the extra water and maintain the needed water inside the body of *Paramecium*.

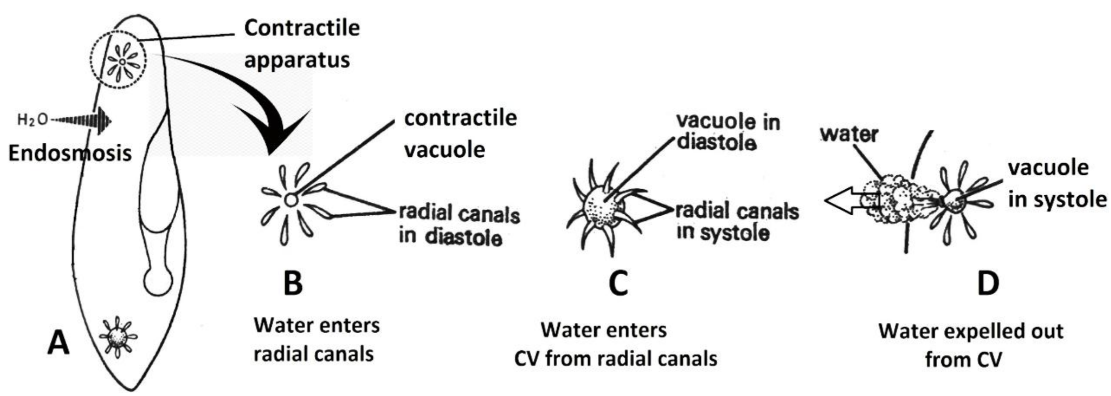

Figure 20. Osmoregulation in *Paramecium*

BEHAVIOUR

Behaviour of an animal involves the responses shown by the individual to its environment. The responses of the animal to different stimuli are known as 'taxes'. The behaviour is determined by the environmental influences or stimuli to which the organism is subjected. The responses shown by *Paramecium* to different stimuli are positive as well as negative. Interestingly, Paramecium is a unicellular animal and has no sense organs.

(a) Avoiding reaction. If a fast swimming *Paramecium* strikes a solid object, it moves back for a short distance, turns on its side and swims forward again but at an angle to its original path. If it again collides with an obstacle, it shows the same negative reaction.

(b) Trial and error reaction. It is seen in various experiments that *Paramecium* can learn to behave under certain circumstances. It continuously tests the water by drawing it in the oral groove. It also checks the temperature of the water and if the water is hot or cold, it can avoid that particular situation by going to some other place. These behavioral patterns help the *Paramecium* to avoid undesirable environmental stresses.

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Responses to different stimuli

1. *Temperature.* Response to temperature is called thermotaxis. The optimum temperature for Paramecium is between 25°C to 28°C. if the temperature declines or elevates, the *Paramecium* shows a negative response. Hence *Paramecium* tries to stay in the water with optimum temperatures.

2. Touch. Response to contact is called thigmotaxis. Whenever the anterior end of *Paramecium* is touched with a solid object, it gives a negative response and moves away from it. If the *Paramecium* is swimming fast and touches a solid object, again it gives a negative response and moves away from it. If the *Paramecium* is moving slowly and experiences a solid object, it comes to rest in contact with the object. It gives a positive response if the solid object is food.

3. Light. Response to light is termed phototaxis. *Paramecium* shows an avoiding reaction to both strong light as well as darkness. Paramecia also move away from ultraviolet rays. Paramecia show a positive response to moderate light. Hence, *Paramecium* tries to remain in dim light and avoids complete darkness or strong light areas.

4. Chemicals. Response to chemicals is called chemotaxis. Responses to chemicals are negative in most of the experimental cases. *Paramecium* tries to avoid both acidic and alkaline environments. In experiments, it has been seen that the animal shows an avoiding reaction to a drop of weak salt solution. On the other hand, *Paramecium* shows a positive response towards a drop of weak acid. *Paramecium* finds and selects their food preferably on the basis of chemotaxis.

5. Water current. Response to water currents is known as rheotaxis. Paramecia show a positive rheotactic response. Paramecia swim against the water current with their anterior blunt end upstream.

6. Electric current. Response to electric current is known as galvanotaxis. A positive galvanotaxis is shown to weak electric current. In experiments, *Paramecium* has been observed to move towards the cathode or the negative electrode. There is a negative response of the Paramecia to a strong current.

7. Gravity. Response to gravity is called geotaxis. Paramecia generally show a negative geotaxis or response to gravity as seen in a culture contained in a test tube, where they gather close to the surface film with their anterior ends pointed upwards.

REPRODUCTION

Paramecium undergoes asexual reproduction by different methods. *Paramecium* also undergoes several kinds of nuclear reorganisations including conjugation, endomixis, autogamy and cytogamy. Under certain conditions of stress, it also undergoes encystment which involves the formation of a cyst wall.

(a) Binary fission (Breaking into two)

During favourable conditions, *Paramecium* commonly reproduces by binary fission. This fission process is transverse in nature as the fissure appears at right angles to the longitudinal axis of the body. Hence, it is also referred to as transverse binary fission. Whenever *Paramecium* has to undergo binary fission, it stops feeding. Its oral groove and buccal structures begin to disappear.

Karyokinesis. It is the division of the nucleus. *Paramecium* has two types of nuclei. Out of these two, the macronucleus undergoes division by amitosis (without spindle formation) whereas the micronucleus divides by mitosis (spindle formation). Micronucleus divides by mitosis and involves all the typical stages of mitosis. In the prophase stage, each chromosome begins to split longitudinally to form two chromatids. In the metaphase, paired chromatids arrange themselves on the nuclear spindle at the equatorial plane or the metaphasic plate in the centre. Then follows the anaphase stage in which the chromatids separate apart to the opposite poles. In the telophase

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stage, the micronucleus becomes elongated and the two ends organise themselves into two daughter micronuclei which soon separate out. Simultaneously, the macronucleus divides amitotically. This process lacks the formation of spindle. In this case the macronucleus simply elongates. A constriction is formed in the centre of the nucleus and is divided into two.

Cytokinesis. It is the division of the cell or the cytoplasm of the cell. It results in two daughter organisms. After the nuclear divisions of micro- and macronuclei, two oral grooves begin to form. One oral groove is formed at the anterior half and the other in the posterior half. Two original contractile vacuoles of the parent Paramecium remain in their respective positions. These vacuoles automatically fall in the two future daughter Paramecia. Two new oral apparatus are formed. Finally, a constriction (furrow) appears in the middle of the body. It gradually deepens from either side and ultimately the animal body or cytoplasm is completely divided into two. It results in two daughter paramecia. Daughter *Paramecium* resulting from the anterior end of the parent is termed "protor" and one from the posterior end is "opisthe". Proctor and opisthe soon become bigger in size and start to lead their individual lives in the medium.

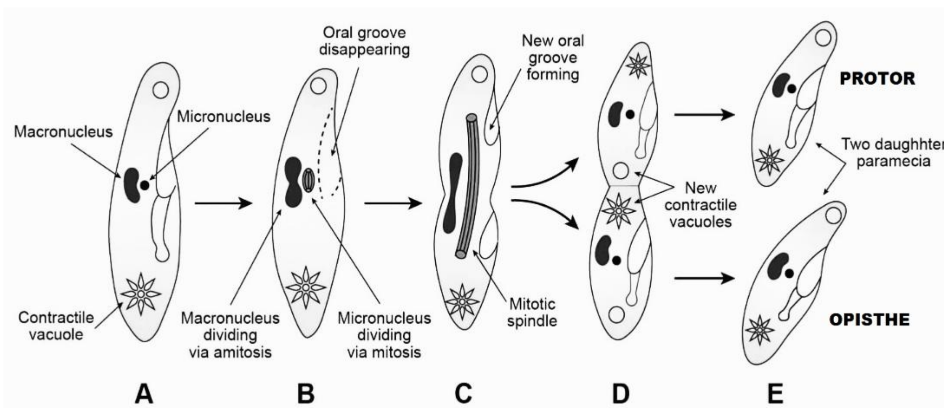

Figure 21. Binary fission in *Paramecium caudatum*

The process of binary fission is completed in about 30 minutes, though separation of daughter paramecia takes about one hour. The term clone is used to refer to all the individuals (genetically alike) that are produced asexually from one parent *Paramecium*.

(b) Conjugation (Sexual phenomenon)

Under certain conditions, such as overcrowding or environmental stress, *Paramecium* turns from strictly asexual reproduction to sexual reproduction. *Paramecium* undergoes a sexual phenomenon called conjugation. This involves the exchange of genetic material between two individuals of different 'mating strains'. In pairing conjugants there is no morphological sexual dimorphism into male and female conjugants. This remarkable process in *Paramecium* occurs frequently between binary fissions and is necessary for the continued vitality of the species.

Mechanism. Through a process called conjugation, two paramecia (preconjugants) line up side by side and then fuse together by their oral sides. They stop feeding and their buccal structures disappear. The pellicle and ectoplasm degenerate at the point of contact and a protoplasmic bridge is formed between the two individuals, which are now called the conjugants. Macronucleus simply breaks up into fragments and is degenerated in both the paramecia. The diploid micronucleus undergoes meiosis and forms four micronuclei. Out of these 4 micronuclei, 3 disintegrate and only one is left. This micronucleus now undergoes mitosis and 2 haploid micronuclei are formed.

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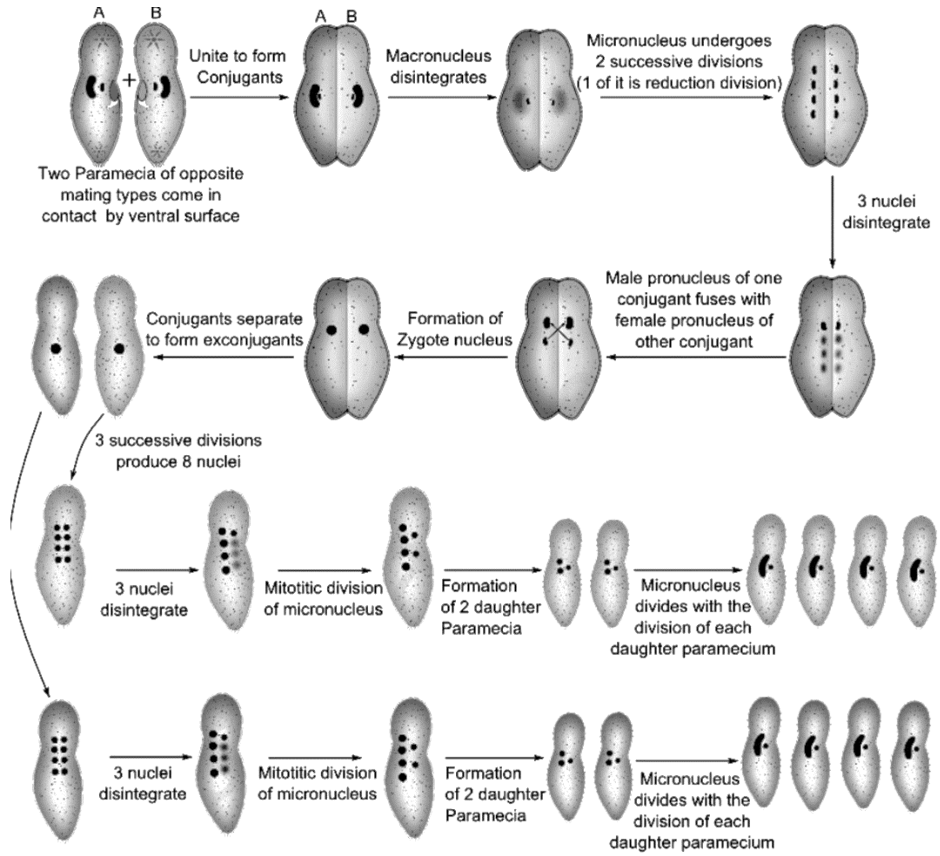

Figure 22. Conjugation in *Paramecium caudatum*

One of the two grows in size and is referred to as the stationary nucleus while the smaller is known as migratory nucleus. The migratory nucleus (N) of one conjugant then passes through the protoplasmic bridge into the other individual and fuses with its stationary nucleus (N), forming a single diploid zygote or synkaryon. The complete fusion of two nuclei from two different individuals forming a zygote nucleus is termed amphimixis. After the formation of synkaryon, two conjugating paramecia separate and are now called exconjugants. In each separated exconjugant, the zygote divides by mitosis three times producing 8 nuclei. Out of these eight nuclei, 4 enlarge to become macronuclei and other 4 become micronuclei. Three micronuclei disintegrate out of 4. The one micronucleus left again divides along with binary fission of the exconjugant. Thus, from each exconjugant two daughter paramecia are obtained, each containing 2 macronuclei and one micronucleus. The micronucleus again divides with the division of each daughter paramecium, forming two individuals each containing one macronucleus and one micronucleus. Each exconjugant produces four daughter individuals at the end of conjugation. Whole process involving 2 conjugants results in the formation of 8 daughter paramecia. It represents a combination of genetic material derived from two genetically different individuals. This chromosome mixing is the basic principle of creating genetic diversity.

Conditions governing conjugation. This method of reproduction is preferred by the animal only in adverse conditions like scarcity of food. Some chemicals can also induce conjugation. Conditions of light and temperature should be optimum for conjugation to occur. These conditions can vary depending upon the species. A minimum level of nutrition must be present. Starved paramecia generally do not undergo conjugation. Individuals must have passed through a desirable number of asexual reproduction. After that sexually mature individuals are formed which further can go for conjugation. For conjugation to happen, two conjugates must be of different mating types. Conjugation never happens among pure lines. Pure lines are the descendants of a single individual and are just like brothers or sisters.

(c) Autogamy

It is a method of nuclear reorganization in P. *aurelia.* This process resembles conjugation. But here in autogamy, only one parent is involved rather than two. This process is also known as self-conjugation. P. *aurelia* has two micronuclei and a single macronucleus. Macronucleus starts to disintegrate and is finally absorbed into the cytoplasm. Two micronuclei (2N) divide by meiosis to form eight daughter nuclei (N). Out of the eight, seven nuclei disintegrate and one is left. This single micronucleus (N) undergoes a mitotic division resulting in two nuclei (N). Now these two gamete nuclei (N), present in a single individual, fuse together to form a zygote nucleus (2N). The zygote nucleus further undergoes 2 mitotic divisions which give rise to 4 nuclei (2N). Out of these 4, two grow in size and form macronuclei. Now, we have 2 micro- and 2 macronuclei in the single individual. Now, the individual divides into two daughter paramecia, each having a macronucleus and two micronuclei.

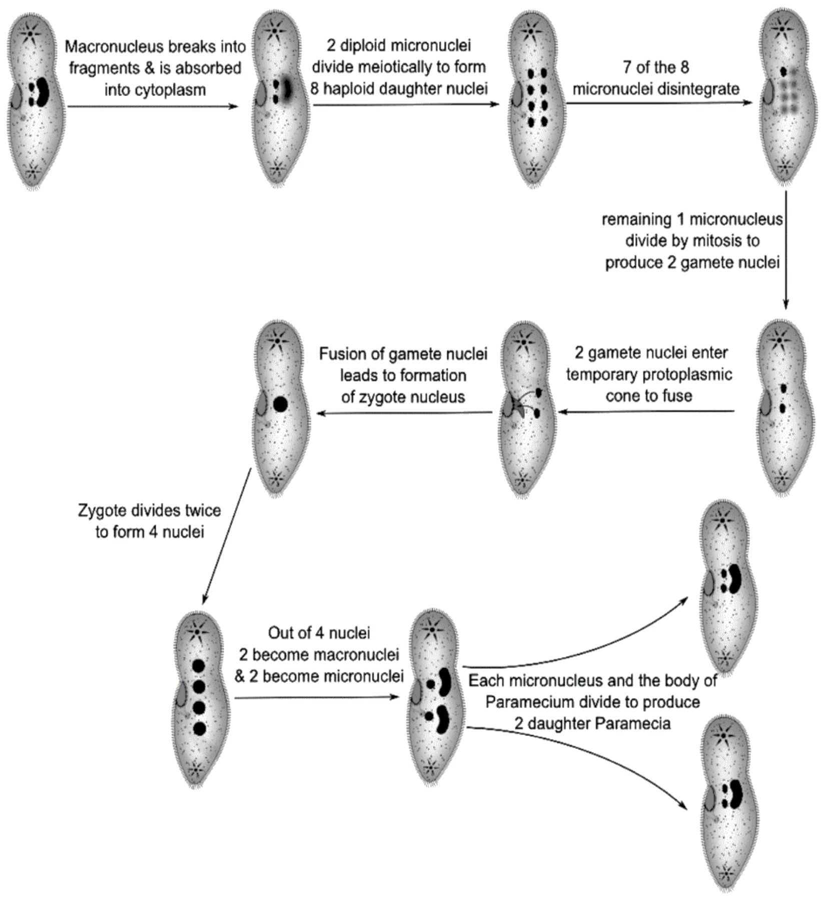

Figure 23. Autogamy in *Paramecium aurelia*

(d) Cytogamy

It is a method of reproduction occurring in P. *caudatum.* It also resembles conjugation as it involves two parents. It is a sexual process without nuclear exchange. The process is completed in about 13 hours. Two paramecia temporarily fuse by their oral surfaces and the nuclear divisions start as in the case of conjugation (not repeated here). The difference in conjugation and cytogamy lies in the way zygote is formed. In conjugation, the migratory nucleus of one parent enters the other parent and fuses with the stationary nucleus to form the zygote. But here in cytogamy, the nuclei fuse with each other in the same parent without migration. Here the two parents are termed "cytogamonts".

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Difference between Conjugation, autogamy and cytogamy

Conjugation is a sexual type of reproduction in *Paramecium* which involves the fusion of the micronuclear material in between the two parents. In this process, the micronucleus or migratory nucleus migrates from one parent to the other to fuse with the stationary nucleus forming a zygote. In the case of autogamy, only one individual is involved and this method is prevalent in P. *aurelia*. The two gamete nuclei fuse within the same individual to form the zygote. In the case of cytogamy, two parent paramecia fuse together as in conjugation but two gamete nuclei fuse inside their respective parents to form the zygote.

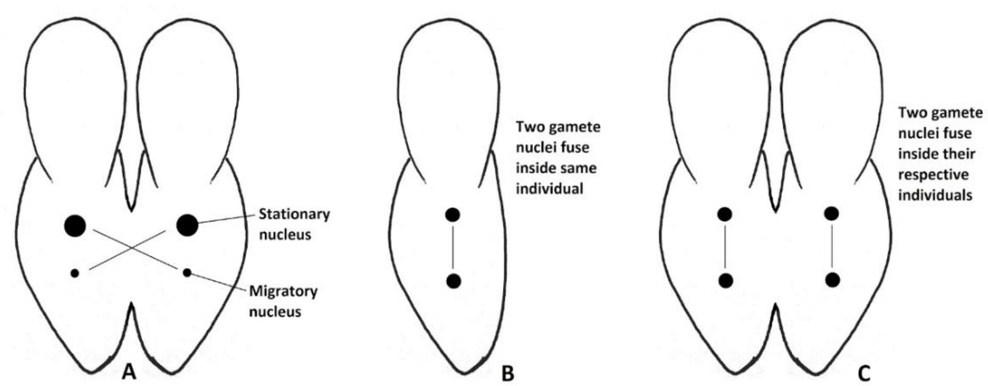

Figure 24. Differences between conjugation (A), autogamy (B) and cytogamy (C)

(e) Endomixis

Endomixis is the process of nuclear reconstruction within the same individual without involving any fusion of the gamete nuclei. It occurs in P. *aurelia*. So it can be considered equivalent to parthenogenesis among the higher animals. This type of reproduction occurs after about 50 generations produced by binary fission as P. *aurelia* do not undergo the process of conjugation.

P. *aurelia* has a single macronucleus and two micronuclei. The macronucleus is not involved in the reproduction process so it is broken up into pieces which are absorbed into the cytoplasm. Both micronuclei undergo two mitotic divisions to form 8 micronuclei. Out of these eight micronuclei, 6 disintegrate and two are left. Then the *Paramecium* divides into two daughter cells, each having one micronucleus. Now this single micronucleus in both the daughter paramecia divides by mitosis twice to produce 4 micronuclei. Out of these 4, two grow in size to form macronuclei. Now each daughter *Paramecium* has two macronuclei and two micronuclei. Afterwards, only the two micronuclei divide by mitosis to form 4 micronuclei. Each daughter *Paramecium* has two macronuclei and four micronuclei. Now the *Paramecium* divides by binary fission resulting in 4 daughter paramecia, each having one macronucleus and two micronuclei. Thus the process of endomixis results in the formation of 4 daughter paramecia from a single individual. This particular process does not involve meiosis or the fusion of gamete nuclei, as we have seen in the previous methods of reproduction.

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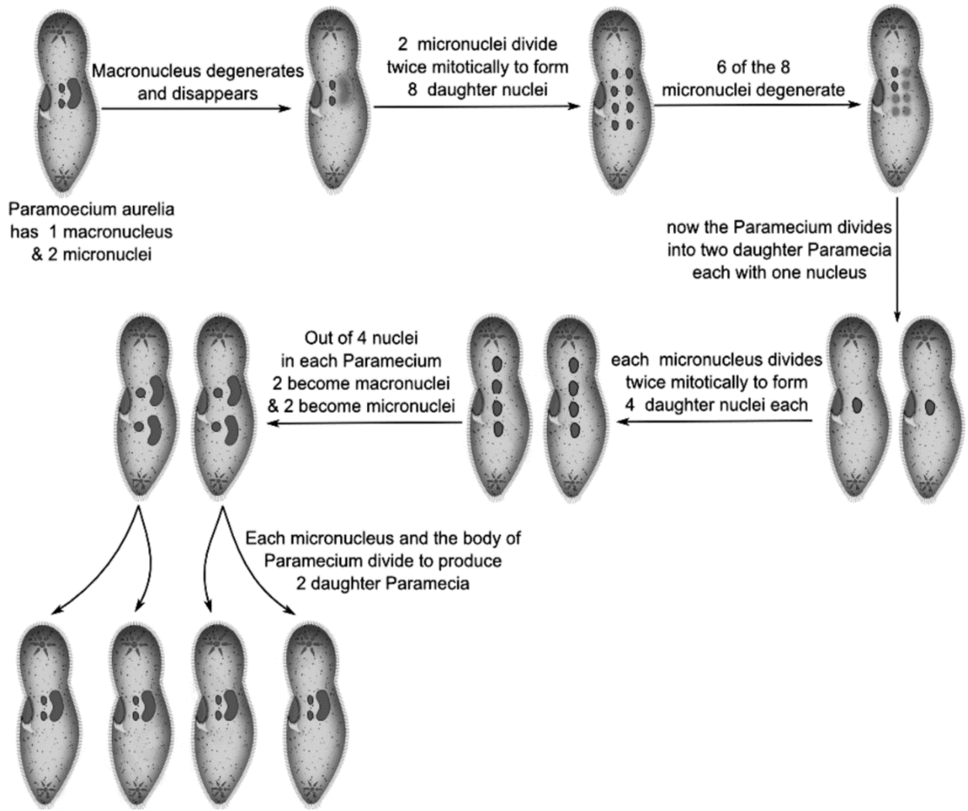

Figure 25. Endomixis in *Paramecium aurelia*

(f) Hemixis

Hemixis involves the reconstruction of only macronucleus without any change in micronuclei. As *Paramecium* grows old, its macronucleus gains more DNA and becomes bigger in size. This extra DNA has to be removed, shed or reduced by *Paramecium* to be normal. Diller (1936 A.D.) explained three types of hemixis in P. *caudatum*:

(1) Type A. The macronucleus divides without the division of the micronucleus or cytoplasm. With the division of grown macronucleus, it regains its original smaller size and the *Paramecium* becomes normal.

(2) Type B. The macronucleus sheds some of its part into the cytoplasm where it gets absorbed. This makes the macronucleus smaller and the *Paramecium* regains its normal activity.

(3) Type C. In this type, both the above events occur simultaneously. The macronucleus divides as well as it sheds some of its chromatin into the cytoplasm. That reduces the load on the macronucleus.

Thus, hemixis leads to the rejuvenation of the organism by reducing the size of the macronucleus with which the *Paramecium* regains its active form. As this process does not involve any kind of nuclear exchange, it does not result in variation.

CYTOPLASMIC PARTICLES IN PARAMECIUM

1. Kappa particles. Kappa particles are the self duplicating cytoplasmic particles found in *Paramecium aurelia*. On the basis of presence or absence of kappa particles, *Paramecium* can have two types of strains. If the *Paramecium* has kappa particles in the cytoplasm then it is said to be the 'killer' strain. if the *Paramecium* lacks the kappa particles it is referred to as the 'sensitive' strain. The killer strain can kill the sensitive strain paramecia by producing a toxic protein called 'Paramecin'.

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There are two conditions which should be fulfilled by the individual *Paramecium* to be a killer:

(a) Minimum number of kappa particles. In various experiments, it has been seen that a *Paramecium* becomes killer only if it has a minimum number of 400 kappa particles inside its cytoplasm. This much number of kappa particles (N= 400) is sufficient to produce effective amounts of paramecin to kill the sensitive strains. As kappa particles are self duplicating, they keep on increasing their number. Thus, *Paramecium* is said to be a killer if it has the minimum number of kappa particles inside it. However, The killer strains remain unaffected in the presence of paramecin. Thus, the killer strain affects only the sensitive strains of the *Paramecium*.

(b) Minimum of one dominant K gene. Kappa particles can be retained only in the individuals which possess at least one dominant gene (K) in the nucleus. Thus, the individuals with genotype 'KK' or 'Kk' can retain the kappa particles and can be the killer paramecia. The sensitive ones have the genotype as 'kk'.

Hence, the paramecia which have a favourable genotype and atleast 400 kappa particles are the members of the killer strain. These can retain kappa particles and can produce enough paramecin to kill the sensitive strain. If an individual has a favourable genotype but has 200 kappa particles, it will not be a killer. But, it will attain the killer trait when the number of kappa particles increases to 400, as they can replicate on their own and increase in number.

Inheritance. Kappa particles may be exchanged in between two paramecia of different strains. Conjugation may take place between killer (KK or Kk) and sensitive (kk) strains whenever they are placed in water free from paramecin. There can be two conditions in conjugation depending upon the time for which the two paramecia join each other or conjugate.

(a) Conjugation for a short period (Nuclear exchange). If the conjugation takes place for less than 3 minutes, there is no exchange of the cytoplasm. So, there is no transfer of kappa particles from the cytoplasm of the killer strain conjugant into the sensitive one. In this case, only the nuclear material is exchanged which decides the genotype of the next generation. Thus, one of the exconjugant paramecia (Kk) derived from the killer conjugant is killer. The exconjugant paramecium (Kk) derived from sensitive remains sensitive.

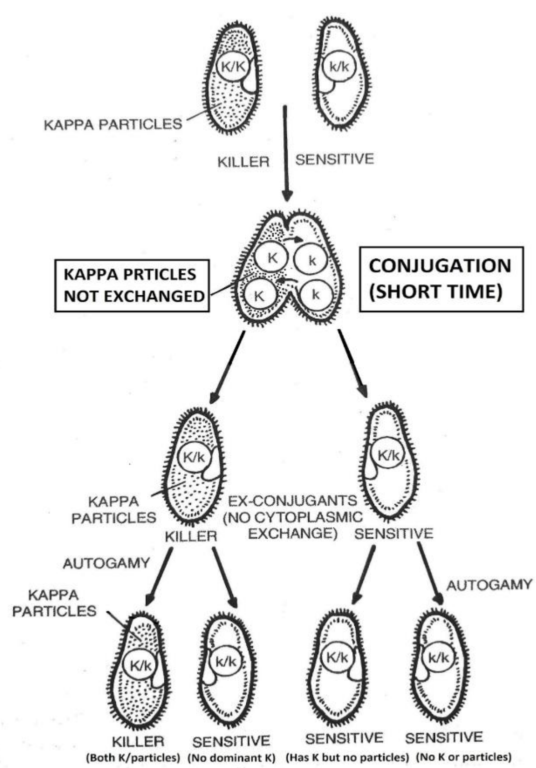

Figure 26. Results of conjugation for a short duration

(b) Conjugation for a long period (Nuclear and cytoplasmic exchange). If the conjugation takes place for a longer time, a complete cytoplasmic bridge is formed. So, there is a transfer of nuclear as well as cytoplasmic material. Kappa particles from the cytoplasm of the killer strain enter the cytoplasm of the sensitive strain. This exchange of kappa particles changes the sensitive strain conjugant into killer strain. The daughter paramecia with at least one dominant gene 'K' become killer as shown in the diagram.

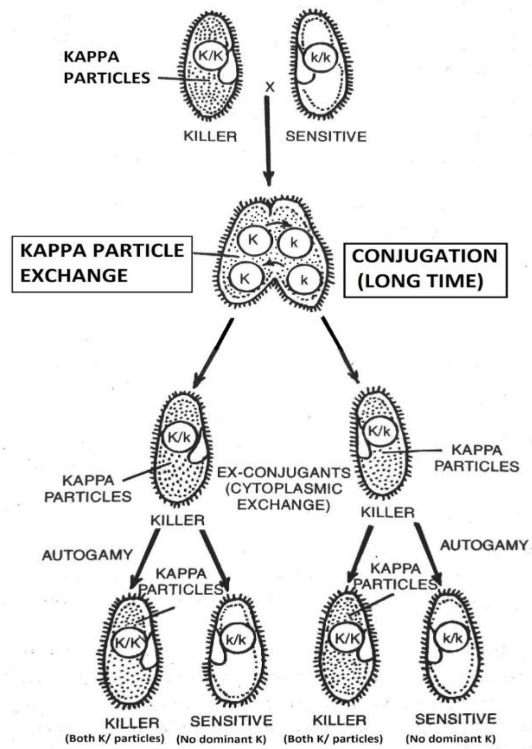

Figure 27. Results of conjugation for a long time period.

2. Pi particles. Pi particles are mutant forms of kappa particles. They do not release any toxic substance meant for killing those which are without such particles.

3. Lambda particles. These particles are present in killer paramecia. These particles are involved in lysing or disintegration of sensitive paramecia.

4. Mu particles. These particles are also present in Paramecium. These particles are involved in killing the mate lacking Mu particles during conjugation.

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