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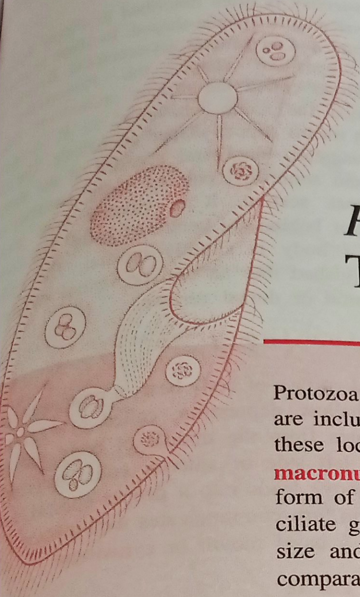
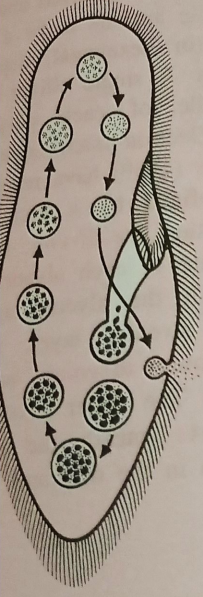
Paramecium caudatum : The Slipper Animalcule



Protozoa which move with the help of **cilia** are called **ciliates** and are included in the subphylum *Ciliophora.* Besides the possession of these locomotor organelles, all ciliates possess two types of nuclei, **macronucleus** and **micronucleus** (nuclear dimorphism), and a unique form of sexual reproduction (**conjugation**). *Paramecium* is a typical ciliate genus containing about 10 known species differing in shape, size and structure. The largest species, *P. caudatum,* has a single comparatively large and compact micronucleus with chromatin material scattered throughout the nucleoplasm. It occurs widely and abundantly. *P. aurelia* has two micronuclei while *P. multimicronucleatum* has many micronuclei. *P. bursaria* is green due to presence of symbiotic alga, **zoochlorella**. In the following text, the biology of *P. caudatum* is treated in detail.



Paramecium caudatum

SYSTEMATIC POSITION

Phylum	Protozoa
Subphylum	Ciliophora
Class	Ciliata
Subclass	Holotricha
Order	Hymenostomatida
Suborder	Peniculina
Genus	*Paramecium*
Species	*caudatum*

OCCURRENCE

Paramecium caudatum (Gr., **paramekes**, oblong + L. **caudata**, tail) is one of the most common species of *Paramecium* having 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 great deal of decaying organic matter. It thrives well in ponds or slowly running streams containing aquatic plants. The paramecia often gather near the surface in scum, but are usually seen actively swimming throughout the water in which they live.

CULTURE OF *PARAMECIUM*

Paramecium is easily grown in wide mouthed jars with glass covers, three-quarter filled with boiled pond water or Chalkey's medium (NaCl 80 mg, $NaHCO_3$ 4mg, KC1 4mg, $CaCl_2$ 4mg, CaH_4 $(PO_4)2H_2O$ 1.6 mg, dissolved in one litre of distilled water), and with 7-12 drops of skimed-milk added weekly. The jars are kept away from direct light to allow bacteria to flourish which serve as food for the multiplying paramecia.

EXTERNAL STRUCTURE

[I] Size

Paramecium is a microscopic, elongated organism which is visible to the naked eye as a whitish or greyish spot. Its species vary in length from 80μ to 350μ. *P. caudatum*, the largest species, measures between 170μ and 290μ. The greatest diameter of the cylindrical body is about two-third of its entire length. *P. aurelia* is about 120μ to 250μ long. Usually the individuals of the same species may exhibit minor morphological and physiological differences. Jennings was able to find in one species of *Paramecium* eight races differing in total length and size.

[II] Shape

Paramecium is often described as slipper shaped, cigar-shaped or spindle-shaped. Its shape is usually constant and in general asymmetrical. Because of its slipper-like shape, the *Paramecium* is sometimes called the **slipper animalcule**. Joblot assigned the name 'chausson' to *P. caudatum* which means slipper-shaped animalcule. The body is elongated, blunt and rounded at the anterior end and somewhat pointed at the posterior end. In cross section, it is circular with greatest diameter behind the centre of body. The anterior half of body is slightly twisted. The body is distinguished into an oral or ventral surface and an aboral or dorsal surface.

[III] Oral Groove

Ventral surface of body bears a prominent, oblique and shallow depression, called **oral groove**. It originates from the middle of body and extends to the left side of anterior end. Posteriorly, the oral groove leads into a deeper conical **vestibule** which in turn communicates with a **buccal cavity** having a basal mouth or **cytostome**.

[IV] Pellicle

External envelope of body is a living, clear, firm and elastic cuticular membrane, the **pellicle**. When stained specimens are observed under light microscope, the pellicle appears to be a regular series of polygonal (or hexagonal) depressions with their raised rims. A single cilium emerges out from the middle of each polygon or circumciliary space. Electron microscopic studies by **Ehret** and **Powers** (1959) have revealed that the polygons are defined by a corresponding regular series of cavities, the **alveoli**. In fact, it is the pit in the centre of each **alveolus** which forms a polygon. All the alveoli collectively form a continuous alveolar layer, which is delimited by an **outer alveolar** and **inner alveolar membrane**. The outer alveolar layer lies in close contact beneath the outer cell membrane (not shown in the diagram). Thus, the

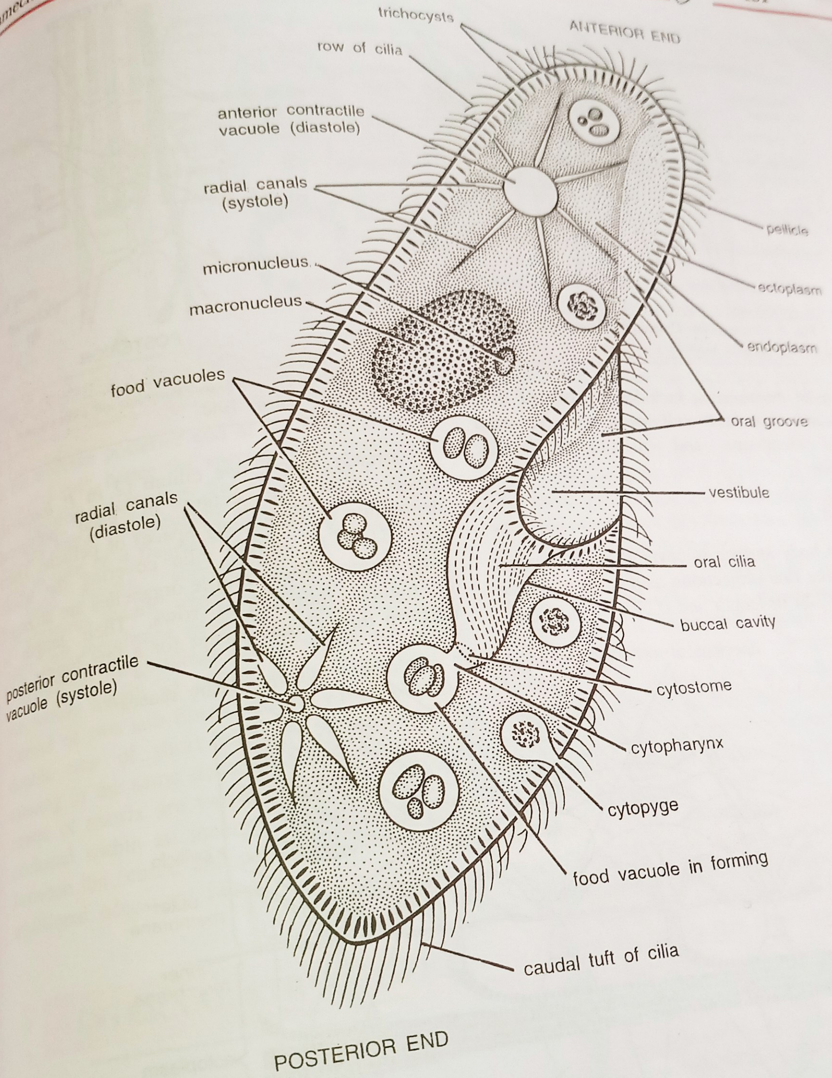


Fig. 1. *Paramecium caudatum.*

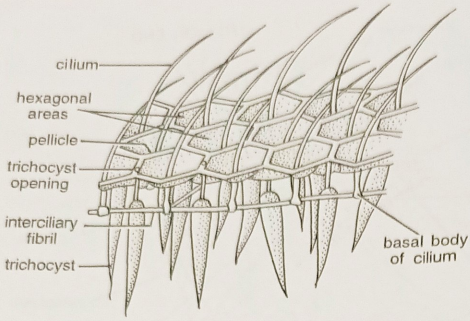


Fig. 2. *Paramecium.* Diagrammatic surface view of a small area of pellicle.

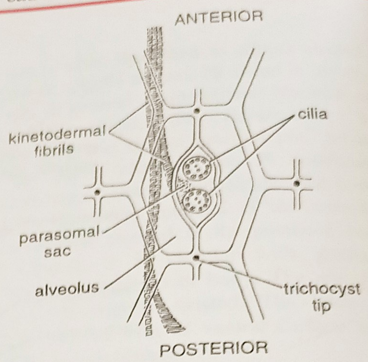


Fig. 3. *Paramecium.* Diagram of a hexagonal area or ciliary field.

pellicle of *Paramecium* includes a series of three membranes : (i) outer cell membrane, (ii) outer alveolar membrane, and (iii) inner alveolar membrane.

[V] Cilia

The entire body surface is covered by numerous, tiny, hair-like fine projections, called **cilia**. These measure 10-12μ in length and 0.27 μ in diameter. As already stated, one cilium (2 in *P. bursaria*) arises from the centre of each polygonal depression (circumciliary space) of pellicle. There are 10,000 to 14,000 cilia covering the whole body surface. These motile organelles are arranged in regular longitudinal rows. Their length is uniform throughout, except for a few longer cilia

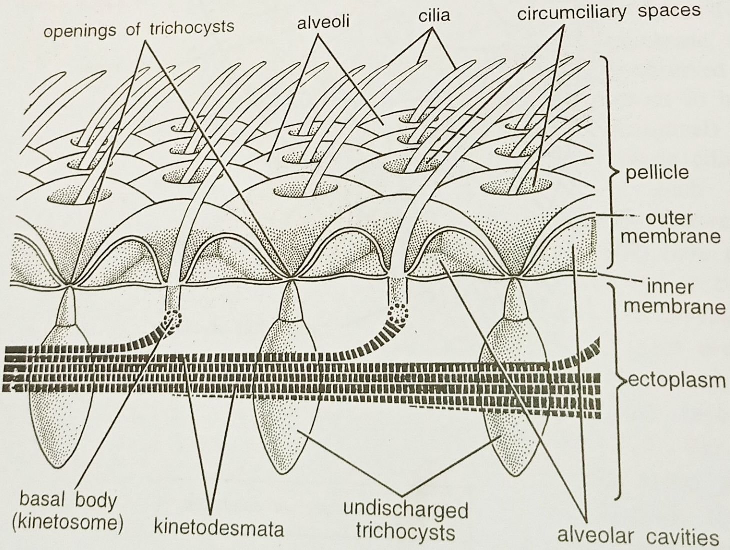


Fig. 4. *Paramecium.* A diagrammatic three-dimensional electron microscopic representation of a portion of pellicle and infraciliary system.

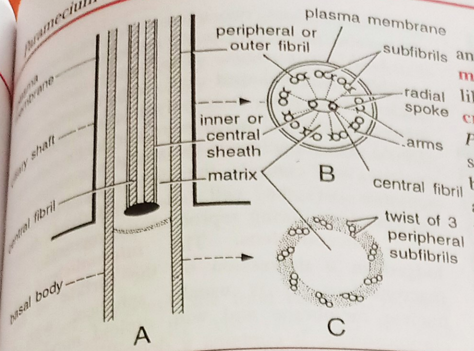


Fig. 5. Structure of a cilium and its basal body. **A.** Cilium in L.S. **B.** Free part of cilium in T.S. **C.** Basal body in T.S.

at the extreme posterior end of the body, forming a **caudal tuft**, hence the species name *caudatum*.

Electron microscopy has shown that a cilium has the same fundamental structure as has been seen in case of a flagellum (Fig. 5). It consists of a fluid matrix, surrounded by an outer membranous sheath, which is continuous with the outer cell membrane of body. Within matrix are 9 peripheral longitudinal fibres, which run along the whole length of cilium body. Each fibre is formed of two sub-fibres, one of which carries a double row of short arms or projections, all running in the same direction (clock-wise). In the centre of matrix are two single fibres, which are enclosed within an inner membranous sheath. In between the central and peripheral fibres are nine additional accessory fibres.

INTERNAL STRUCTURE

[I] Cytoplasm

Within pellicle, the cytoplasm of body is clearly differentiated into two regions.

1. Ectoplasm. The narrow, peripheral, clear and dense zone is called the **ectoplasm** or **cortex**. It includes the structure of the infraciliary system and the trichocysts.

2. Endoplasm. The large, central, granular and semi-fluid zone is the **endoplasm** or **medulla**. It includes the usual cell components like **mitochondria**, **Golgi bodies**, **ribosomes**, **crystals**, **reserve food granules**, etc. In *P. bursaria*, the endoplasm is filled with symbiotic *Zoochlorella*, a unicellular chlorophyll-bearing alga. Prominent endoplasmic inclusions are **nuclei**, **contractile vacuoles** and **food vacuoles**.

[II] Infraciliary System

Immediately beneath the pellicular alveoli is located the infraciliary system constituted by the basal bodies and kinetodesmata.

1. Basal bodies. The base of each cilium is produced into a tube-like structure, called **basal body** or **kinetosome**. It is formed by the thickened basal ends of peripheral fibres of cilium. The central fibres do not enter into it. The wall of basal body consists of 9 triplets of sub-fibres. The basal bodies are self duplicating units and progenitors of new cilia. Each basal body is either a centriole or its derivative.

2. Kinetodesmata. Associated closely with basal bodies of cilia and lying in the ectoplasm is a system of specialized striated fibrils, called **kinetodesmal fibrils**. A single fibril or **kinetodesmos** arises from the kinetosome or basal body of each cilium and runs anteriorly somewhat tapering along the course. It joins its counterparts from the posterior kinetosomes, forming a bundle of overlapping longitudinal fibrils, called **kinetodesma** (pleural, **kinetodesmata**). The number of fibrils in each kinetodesmata constantly remains (5), because the individual fibrils do not run anteriorly farther than 5 basal bodies. It has been suggested that fibrils coordinate ciliary beat and movement, but the evidence is very conflicting.

The kinetosomes of a longitudinal row plus their kinetodesmata constitute a structural unit, called the **kinety**. A kinety system is apparently characteristic of all ciliates. It is said that the pattern of infraciliature plays an important role in the morphogenesis of Protozoa. 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.

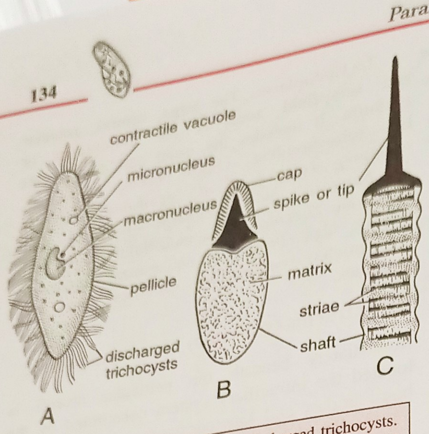


Fig. 6. *Paramecium.* **A.** Animal with discharged trichocysts. **B.** Undischarged trichocyst. **C.** Apical portion of discharged trichocyst.

[III] Trichocysts

Trichocysts are peculiar rod-like or oval organelles present throughout the ectoplasm alternating with basal bodies and oriented at right angles to the body surface. These were first seen in *Paramecium* by Elis. These are very small in size, measuring about 4*μ* in length. Each trichocyst consists of an elongated **shaft** and a terminal pointed **tip**, called the **spike** or **barb**, covered by a **cap**. The matrix of shaft consists of a dense mass of a fibrous protein, called **trichinin**. Its fibres remain condensed forming a cross-striated lattice work.

Function of trichocysts is not well known. It is believed that these discharge and anchor the animal to a firm substratum when it feeds upon bacteria. Others believe that these are organelles of defence.

Discharge of trichocysts is triggered by mechanical, chemical or electrical stimulation. It occurs in a span of a few milliseconds. When fully discharged the shaft becomes a long cross-striated rod and measures about 40μ in length. It is believed that the discharge process consists of an unfolding of the lattice of trichinin fibres.

[IV] Nucleus

Paramecium is **heterokaryotic** as it possesses two types of nuclei. In *P. caudatum*, there is a large **macronucleus** and a small **micronucleus**. Besides the macronucleus, two micronuclei are present in *P. aurelia* and many in *P. mutimicronucleatum*.

1. Macronucleus. The **macronucleus** is roughly kidney-shaped and with inconspicuous nuclear membrane. It is polyploid and possesses many nucleoli and much more chromatin material (DNA). Macronucleus is the somatic or vegetative nucleus and controls the day-to-day metabolic activities of the cell. It is derived from micronucleus during reproductive processes.

2. Micronucleus. The **micronucleus** is lodged in a depression on the surface of the macronucleus. It is usually spherical, with a nuclear membrane and with diploid number of chromosomes. It contains a definite nucleolus in *P. aurelia*, while in *P. caudatum* the nucleolus is absent. It controls the reproductive activities of the organism.

[V] Contractile Apparatus

In *Paramecium*, unlike *Amoeba* and *Euglena*, there are two **contractile vacuoles**, occupying somewhat fixed positions in endoplasm. One vacuole lies near each end of body, close to the dorsal surface. Each of them is surrounded by a circlet of 6 to 10 long, narrow, spindle-shaped **radial canals** (afferent pulsating canals) extending far into cytoplasm. Each contractile vacuole opens to

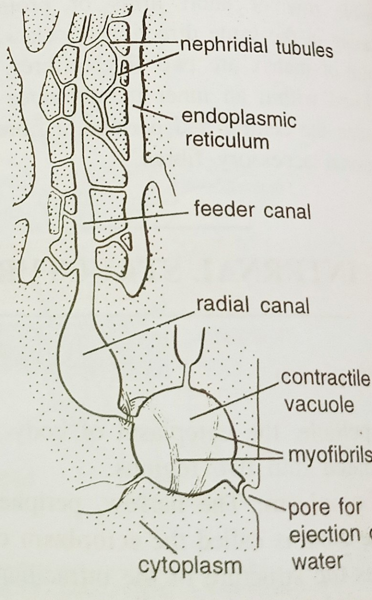


Fig. 7. *Paramecium.* Contractile apparat

outside through a permanent pore in pellicle of dorsal side of body. The two contractile vacuoles do not lose their identity when water is expelled. Electron microscopy has revealed that each contractile apparatus includes some of the tubules of the endoplasmic reticulum, nephridial tubules, feeder canals, accessory vacuoles (radial canals) and the main contractile vacuole. The accessory vacuoles or radial canals are, in fact, the ampullae of feeder canals.

[VI] Food Vacuoles

Numerous non-contractile food vacuoles, recently termed **gastrioles** by **Vokovsky**, can be seen moving with the streaming endoplasm (cyclosis). They differ in shape and size according to the nature of ingested food particles, but mostly they are rounded in form.

[VII] Oral Apparatus

In *Paramecium*, oral groove leads ventrally and posteriorly as a tubular structure, called **vestibule**. It leads directly into a wide tubular passage, the **buccal cavity**. In its turn it opens into a narrow gullet or **cytopharynx** through a narrow aperture, the **cytostome**. The cytopharynx, at its proximal end, forms a **food vacuole**.

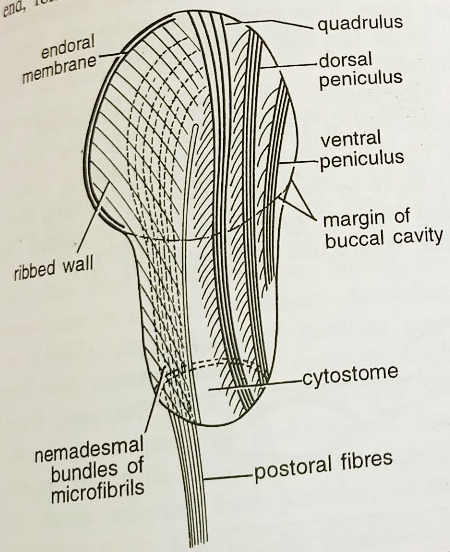


Fig. 8. *Paramecium*. Oral apparatus showing buccal ciliature.

Buccal cavity, at right side, is bordered by a row of cilia forming the **endoral membrane**. At left side are three groups of four rows of cilia, extending from the rim of the opening to the posterior end of buccal cavity. These are **ventral peniculus**, **dorsal peniculus** and **quadrulus**. These ciliary rows constitute the **membranelles**. From endoral membrane a ribbed pellicle extends upto cytostome. **Nemadesmal fibres** run dorsal to the ribbed pellicle and extend as **post-oral fibres** around cytopharynx. Rows of normal somatic cilia line the wall of vestibule.

[VIII] Cytopyge

Near posterior end of body, a little behind cytostome and a little to the right side, a small portion of ectoplasm and pellicle is somewhat weak. Here, at the time of **egestion**, a minute aperture called **cell anus**, **cytopyge** or **cytoproct**, is visible. It is, however, difficult to say whether it is a permanent opening with tightly-closed lips or a temporary opening formed at the time of egestion.

LOCOMOTION

Paramecium has a streamlined body which enables it to swim about in water with a minimum amount of friction. The rapid swimming is facilitated by the beating of fine and hair-like cellular organelles, called **cilia**, that cover the animal's entire cell-body. *Paramecium* moves with a speed of 1500μ or more per second.

[I] Ciliary Beats

During movement, a cilium oscillates like a pendulum. Each oscillation comprises a fast **effective stroke** and a slow **recovery stroke**. During the **effective stroke** or the strong backward lash, the cilium becomes slightly curved and rigid and strikes the water like an oar, so that body is propelled forward in opposite direction of stroke. The **recovery stroke** which follows immediately brings the cilium again into position for the next effective stroke (Fig. 10).

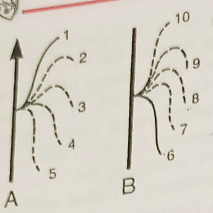


Fig. 9. Diagrams illustrating ciliary movements of a single cilium (after Gray). **A.** Effective stroke. **B.** Recovery stroke.

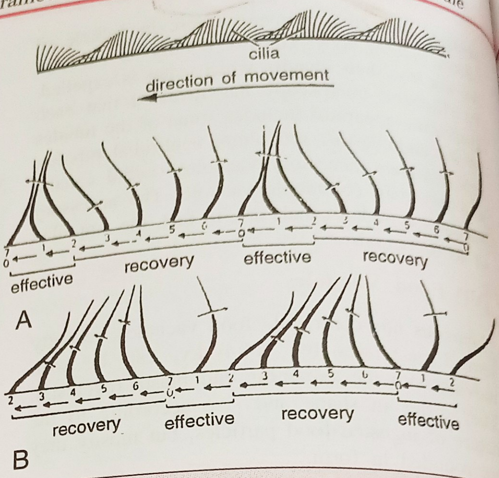


Fig. 10. A. A single row of cilia showing metachronal rhythm. **B.** Cilia 1-7 indicate recovery stroke. Cilia 8-12 indicate effective stroke.

All the cilia of body do not move simultaneously and independently but progressively in a characteristic wave-like manner, called **metachronal rhythm**. The cilia in a longitudinal row beat in a characteristic wave beginning at the anterior end and progressing backwards. Consequently, a cilium in a longitudinal row always moves in advance of the one behind it. All the cilia of a transverse row beat simultaneously or synchronously. During forward movement of *Paramecium* the metachronal waves pass from the posterior end forwards.

[II] Mode of Swimming

The animal does not follow a straight tract but rotates spirally like a rifle bullet along a left-handed helix. The reason for this is twofold. Firstly, the body cilia do not beat directly backwards but somewhat obliquely towards right, so that the animal rotates over to the left on its long axis. Secondly, the cilia of oral groove strike obliquely and more vigorously so as to turn the anterior end continually away from the oral side and move in circles. The combined effect causes the movement of animal along a fairly straight path, rotating about its axis in an anticlockwise direction.

In backward movement a *Paramecium* follows a straight course. In this case the metachronal wave passes from anterior end backwards. This is due to the fact that effective stroke is carried out anteriorly.

Mechanism of ciliary movement in *Paramecium* is little studied. It is now known that cilia are moved in a coordinating system. They move by the contraction of peripheral fibres located within them. The energy needed for fibrillar contraction is supplied by ATP.

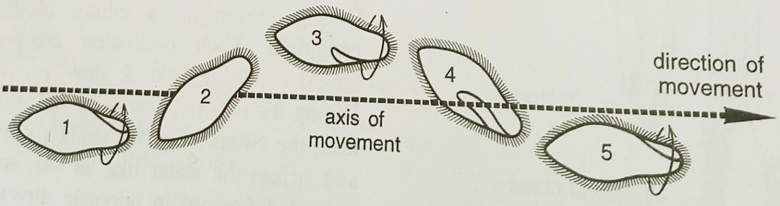


Fig. 11. Anticlockwise spiral path followed by a swimming *Paramecium*.

NUTRITION

[I] Food

Paramecium feeds in the **holozoic** manner, like *Amoeba*. The food consists chiefly of bacteria which float in water in which it lives. It has been estimated that 2 to 5.5 million individuals of *Bacillus coli* are devoured in 24 hours by a single *Paramecium*. In a sense, paramecia are also beneficial to bacteria, lest they might reproduce too rapidly as to endanger their own existence by overcrowding. It also feeds upon small Protozoa, unicellular plants (algae, diatoms, yeasts, etc.) and small bits of animals and vegetables. It will reject most of the non-digestible material and devour certain kinds of food. One species, *P. bursaria*, is interesting, being green in colour due to the presence of numerous unicellular alga, the symbiotic *Zoochlorella* in its endoplasm. It can thus live holophytically for long periods on food substances manufactured by *Zoochlorella*. During scarcity of food, it can digest even its own *Zoochlorella* and can live apparently indefinitely without them.

[II] Feeding Mechanism

Paramecium swims to places where it can get its food. Its food catching apparatus is much more specialized than that of *Amoeba* and *Euglena*.

Food is ingested by a definite cell mouth or **cytostome** lying at the bottom of buccal cavity. The constant lashing movements of cilia of oral groove drive a current of water with food particles towards the vestibule. Ciliary tracts of vestibule direct the food particles into buccal cavity. *Paramecium* is a selective feeder. According to **Mast** (1947), many kinds of particles may be carried with water current into vestibule, but only selected ones are passed on inside the buccal cavity. Rest of particles are rejected, that is, discharged to outside. Passage along which ciliary action drives selected food particles, is termed the **selection path**, whereas passage along which unwanted food particles are driven outside vestibule, is the **rejection path**.

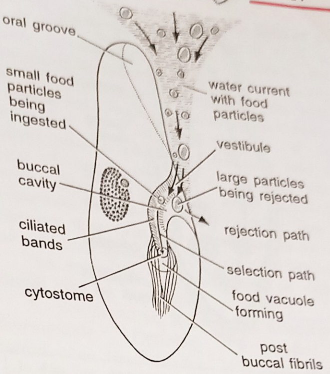


Fig. 12. *Paramecium* receiving food particles with water current drawn into buccal cavity by ciliary action.

Beating of cilia of membranelles of buccal cavity drives the selected food particles through cytostome into cell gullet or cytopharynx. The food now gradually collects at the bottom of cytopharynx into a membranous vesicle which is later nipped off as a **food vacuole**. Another food vacuole may be formed within 1 to 5 minutes depending upon the supply of food and the rate of feeding.

[III] Digestion

Each food vacuole consists of food particles surrounded by a thin film of water. Rapid and irregular movement of endoplasm does not occur in *Paramecium*, but the food vacuole is circulated around the body along a more or less definite path by a slow streaming movement of endoplasm, known as **cyclosis**. Several vacuoles may be seen thus circulating in a definite direction in the endoplasm of a well-fed *Paramecium*. The vacuoles are carried first posteriorly, then forward and aborally and again posteriorly and orally up to cytopyge. Digestion and assimilation of food take place during this journey. Digestive enzymes (proteases, carbohydrases, lipases) are secreted by

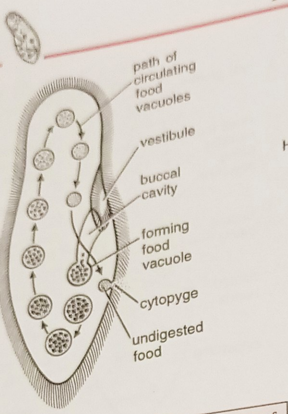


Fig. 13. *Paramecium* showing cyclosis and path of food vacuoles in endoplasm.

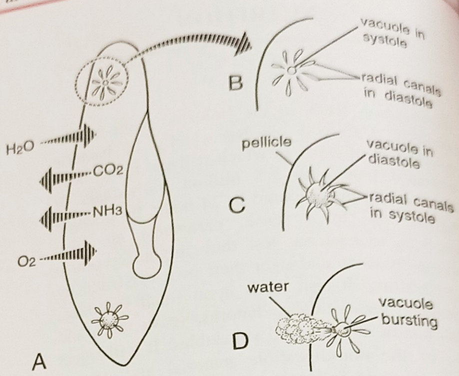


Fig. 14. *Paramecium.* Diagrammatic representation of respiration, excretion and osmoregulation.

the lysosomes into the food vacuoles. As in *Amoeba,* the contents of a vacuole first become increasingly acidic, but later gradually become alkaline. This can be demonstrated with the help of Congo Red and other indicator dyes. The alkaline phase results from the secretion of enzymes within an alkaline medium into the vacuole. Products of digestion (glycogen and fat globules) are diffused into the surrounding cytoplasm and either stored or used for vital activity and growth.

[VI] Egestion

The vacuole gradually becomes smaller as digestion and absorption proceed. Finally, the undigested residual matter is eliminated from body, through a definite **anal spot** or **cytopyge** on ventral surface, posterior to cytostome. The cytopyge is of the nature of a potential cell anus as the undigested matter is always discharged at this spot.

RESPIRATION AND EXCRETION

Respiration takes place, as in *Amoeba* and other freshwater Protozoa, by diffusion through the semi-permeable pellicle. Oxygen dissolved in water is diffused in and used for oxidation of protoplasmic molecules. Catabolic waste products such as CO_2 and nitrogenous matter (NH_3) simply diffuse out into external water because their concentration is always higher in body. Crystals present in cytoplasm are in fact excretory products, which get dissolved and eliminated with the fluid of contractile vacuoles.

OSMOREGULATION

The function of the contractile vacuoles in *Paramecium* is **osmoregulation**.

An excess of water accumulates in body because of continuous endosmosis, the concentration of body cytoplasm being higher than that of external medium. Small quantities of water are also taken in along the ingested food. This excess of water is got rid off by means of contractile vacuoles which contract (**systole**) and expand (**diastole**) at regular intervals, assisted by the contractility of **myofibrils** (see Fig. 7).

Water from cytoplasm is secreted into some of the tubules of endoplasmic reticulum from where it flows down the nephridial tubules into feeder canals to accumulate in latter's ampullae (radial canals). The ampullae converge and discharge into contractile vacuole. When vacuole has grown to its maximum size, it contracts and discharges to the exterior, through a pore in pellicle on dorsal side. Posterior contractile vacuole pulsates faster than anterior vacuole because of the large amount of water being delivered into posterior region by cytopharynx.

BEHAVIOUR

The way in which an organism establishes an active relationship to its environment is called **behaviour**. It is largely determined by the environmental influences or **stimuli** to which the organism is subjected. The responses of *Paramecium* to various kinds of stimuli, such as light intensity, temperature, concentration of O_2, CO_2 and different chemicals in water are interesting. These produce definite behavioural patterns or **reactive behaviour**. The response is **positive** if the animal moves towards a stimulus, and **negative** when it moves away.

Avoiding Reaction

It is perhaps the most important mode of behaviour exhibited by *Paramecium*. If a fast swimming individual 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 which is repeated until the animal passes the obstacle or becomes exhausted.

Trial and Error Reaction

Paramecium can also learn by **trial** and **error reaction**, involving a series of experiments on the part of animal. It constantly tests water just ahead by drawing it in its oral groove in the form of a cone. If water is too hot or too cold or if it contains an irritating chemical substance, the animal shows an avoiding reaction. It immediately backs up and pivots upon its posterior end, while the anterior end swings in a circle. Again it swings forward but in a different direction. If this avoiding reaction once more brings it into the region of the stimulus, it is repeated. These reactions help the animal to avoid undesirable environment without actually getting into it. Moreover, these bring the animal sooner or later, into the most favourable part of environment.

The responses of *Paramecium* to different stimuli may be summarised as under :

1. Temperature. Response to temperature is **thermotaxis.** Optimum temperature for *Paramecium* lies between 24°C and 28°C. An avoiding reaction is given to the temperatures higher or lower than this, until the animals escape or get killed.

2. Light. Response to light is **phototaxis.** Paramecia do not respond to ordinary changes of light, but a negative response is shown to strong light, darkness and ultra-violet rays.

3. Touch. Response to contact or **thigmotaxis** is variable. If the more sensitive anterior end is strongly touched with a solid object, the avoiding reaction is given. But a slow-moving *Paramecium* frequently comes to rest in contact with an object, such as an alga or a plant stem, which can provide rich supplies of food.

4. Chemicals. Chemotaxis or response to chemicals is negative in most of the cases. The animals show a definite avoiding reaction and do not enter a drop of weak salt solution. However, a positive reaction occurs with a drop of weak acid solution. The animals also find and select their food in this manner.

5. Water current. Paramecia show a positive **rheotaxis**, orienting themselves with their anterior ends upstream and swimming against the current.

6. Electric current. A positive **galvanotaxis** is shown to weak electric current, the animals moving towards the negative pole (cathode). A strong current, however, causes them to move backward towards the anode, finally to disintegrate and die.

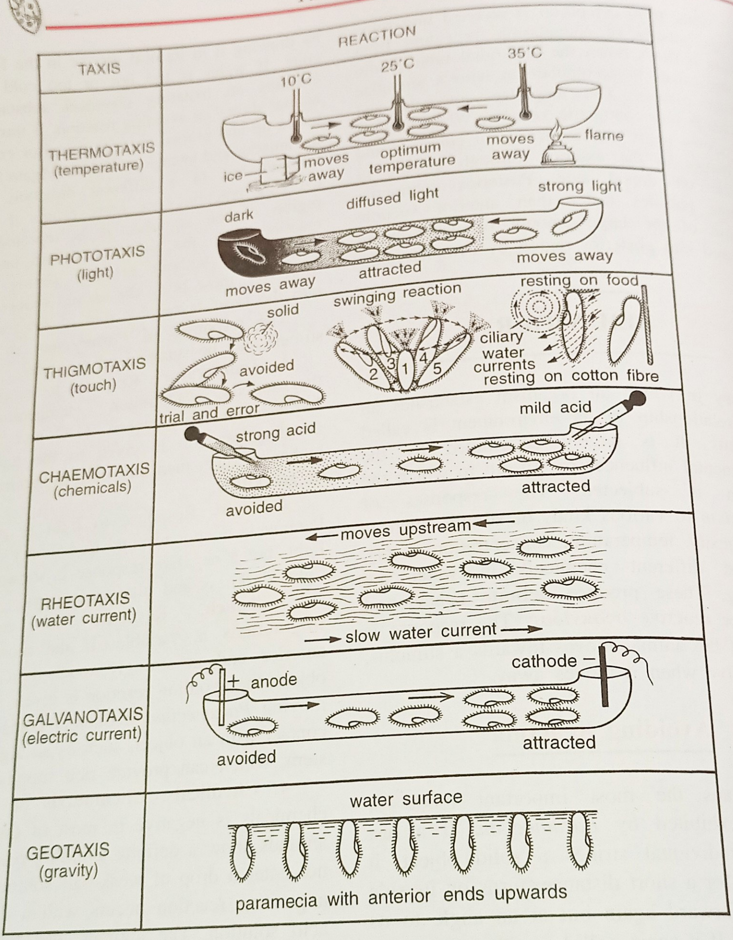


Fig. 15. *Paramecium.* Responses to different stimuli.

7. Gravity. 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. If paramecia are introduced in an inverted water-filled U-tube stoppered at both ends, they immediately move upward into the horizontal part of the tube. When, in moving across the tube, they find their path going downward, they reverse their direction of movement.

REPRODUCTION

Paramecium reproduces asexually by transverse binary fission and also undergoes several kinds of nuclear reorganisations, such as conjugation, endomixis, autogamy, etc. Under certain conditions of food and temperature, it undergoes encystment.

Transverse Binary Fission

During favourable conditions, *Paramecium* commonly reproduces by transverse or horizontal **binary fission**, which is at right angles to the longitudinal axis of body. *Paramecium* stops feeding and its oral groove and buccal structures begin to disappear. While this is happening, the **micronucleus** starts dividing by the complicated process of mitosis, the nuclear membrane remaining intact. Micronucleus first increases slightly in size and then chromosomes, numbering from 36 to 150, depending upon the race, begin to appear. Each chromosome splits longitudinally to form two chromatids (**prophase stage**). Paired chromatids now get arranged on the nuclear spindle at its equatorial plane (**metaphase stage**). This is followed by separation apart of chromatids and elongation of micronucleus (**anaphase stage**). By the last stage (**telophase stage**), micronucleus becomes very much elongated and its two ends become organised into two daughter micronuclei. The daughter micronuclei then separate. Simultaneously, the **macronucleus** divides amitotically by simply becoming elongated and constricted in the middle. Two oral grooves now begin to form, one in the anterior half and the other in the posterior half. Two original contractile vacuoles remain, one in each half of the dividing parent individual. Two new contractile vacuoles are later formed. Two new buccal structures also appear. In the meantime, a constriction furrow appears near middle of the body. It deepens and ultimately the cytoplasm is completely divided, resulting into two daughter paramecia. Of the two daughter paramecia, the anterior one is called **proter** and the posterior, **opisthe**. These grow to full size and divide again by fission.

P. caudatum divides 2-3 times in a day by binary fission. The process is completed in about 30 minutes, though separation of daughter paramecia takes about one hour or more. The term **clone** is used to refer to all the individuals that are produced asexually from one parent *Paramecium*. All the members of a clone are genetically alike.

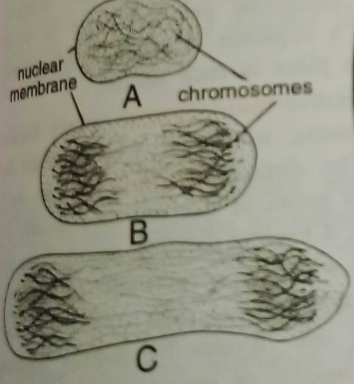


Fig. 16. *Paramecium*. Chromosomal movement in mitosis of micronucleus.

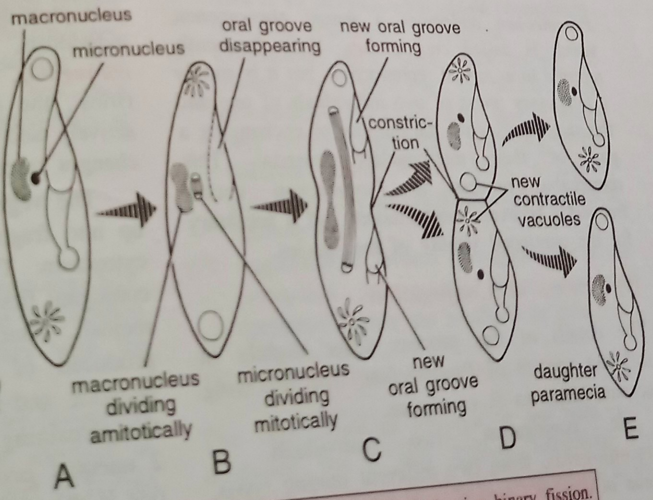


Fig. 17. *Paramecium*. Stages showing binary fission.

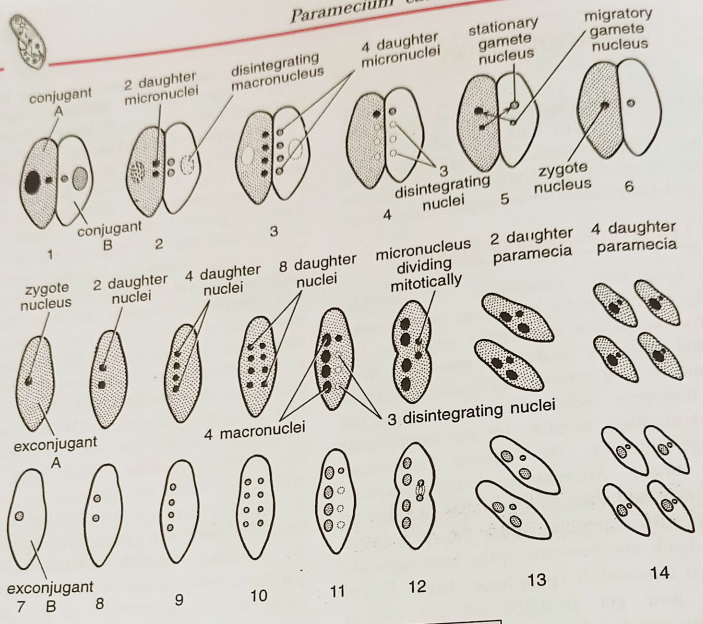


Fig. 18. *Paramecium.* Stages in conjugation.

Conjugation

Paramecium undergoes a sexual phenomenon, which is called **conjugation**. It is frequently referred to as sexual reproduction, but it is simply a temporary union of two individuals of one and the same species for the purpose of exchanging a part of their micronuclear material. This remarkable process in *Paramecium* occurs frequently between binary fissions and is necessary for the continued vitality of the species.

[I] Process of Conjugation

The details of this process differ slightly in different species of *Paramecium.* The following account refers to *P. caudatum.*

In conjugation, two individuals or **preconjugants**, from two different mating types, come in contact ventrally and unite by their oral grooves. 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**. While so united, like the 'Siamese twins', the conjugating pair continues to swim actively and a sequence of complicated nuclear changes takes place in each animal.

The vegetative **maeronucleus** simply breaks up into fragments, which are later absorbed by cytoplasm. The diploid **micronucleus** of each conjugant first grows in size and then divides by meiosis. Thus, 4 haploid daughter micronuclei are produced of which 3 degenerate or become pycnotic and disappear in each conjugant, while the remaining one divides by mitosis forming 2 unequal **pronuclei** or **gamete nuclei**. Smaller one is the active **migratory gamete nucleus** and the bigger one is the passive **stationary gamete nucleus**. The migratory nucleus of one conjugant

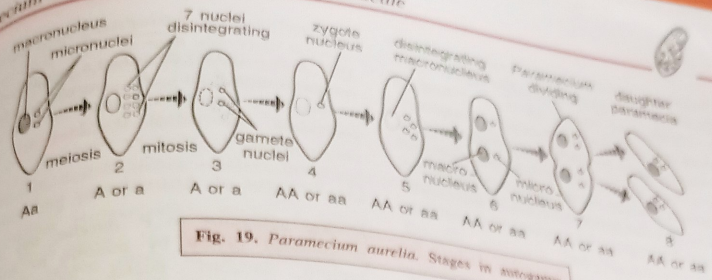


Fig. 19. *Paramecium aurelia*. Stages in autogamy.

then passes through the protoplasmic bridge into the other individual and fuses with its stationary nucleus, forming a single diploid **zygote nucleus** or **synkaryon**. The complete fusion of two nuclei from two different individuals forming a zygote nucleus is termed **amphimixis**.

The two pairing paramecia, after a union of about 12 to 48 hours, separate and are now called **exconjugants**. In each exconjugant, the zygote nucleus divides by mitosis three times in rapid succession producing 8 nuclei, of which 4 enlarge to become **macronuclei** and other 4 become **micronuclei**. Three micronuclei disintegrate and disappear, while the remaining micronucleus divides, with binary fission of 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. Thus, each conjugant produces four daughter individuals at the end of conjugation.

[II] Factors and Conditions of Conjugation

Conjugation is very complex physiologically. The factors and conditions governing conjugation are several and these may also vary with the species.

(1) Conjugation does not occur under favourable living conditions. Starvation or shortage of food and a particular bacterial diet or certain chemicals are said to induce conjugation in some species.

(2) A certain range of light and temperature, differing with species, is said to be essential for conjugation to occur.

(3) In *P. caudatum*, conjugation usually starts early in morning and is continued till afternoon.

(4) The conjugating individuals are usually smaller in size (210μ long) than the normal individuals (300-350μ long).

(5) A definite state of nutrition is indispensable since starved or overfed individuals generally will not conjugate.

(6) Maupas maintains that individuals must have passed through a desirable number of asexual generations (period of immaturity) before they become sexually mature and conjugate.

(7) The pairing conjugants are **isogamous** and there is no morphological sexual dimorphism into male and female conjugants.

(8) Conjugation never takes place among the members of a 'pure line', that is among the descendants of a single individual. It occurs only between individuals belonging to two different **mating types**. Thus, a sort of physiologically sexual differentiation exists in *Paramecium*.

(9) Agglutination favours conjugation. It is the interaction of mating type substances (proteins) which are localized in cilia.

[III] Significance of Conjugation

The significance of conjugation has been much discussed but it still remains uncertain. The following functions or effects are attributed to this process :

1. **Rejuvenation.** If binary fission continues repeatedly for several generations, the *Paramecium* loses its vigour and enters upon a period of depressed physiological efficiency and senescence. The individual ceases to multiply, reduces in size, degenerates in organization and eventually dies off. To avoid this senile decay of race, conjugation is resorted to and the process seems to rejuvenate and revive the lost vigour for asexual reproduction.

However, **Woodruff** and **Jennings** do not support the view that conjugation helps in rejuvenescence. Woodruff succeeded in maintaining a culture of paramecia for nearly 36 years, resulting in hundreds of thousands of generations without resort to conjugation.

2. **Nuclear reorganization.** During conjugation the nuclear apparatus is reorganized and a readjustment occurs between it and the cytoplasm. Probably the macronucleus loses its potentialities in performing its manifold metabolic activities. Its replacement by a new macronucleus brings renewed vigour and vitality to accelerate the metabolic activities.

3. **Hereditary variation.** During asexual reproduction by fission, the hereditary material of the parent passes unchanged on to the progeny, so that all the descendants of one *Paramecium* have the same inheritance. The periodic occurrence of conjugation, however, ensures **inherited variation**. It brings about the blending of two lines of ancestry just as bisexual reproduction does.

[IV] Genetic Consequences of Conjugation

If conjugation takes place between two paramecia, one homozygous for a dominant gene (AA) and the other homozygous for its recessive gene (aa), the first generation would be heterozygous (Aa). If the two conjugants are already heterozygous (Aa), then the resulting progeny would be either homozygous or heterozygous, depending upon which gene gets eliminated at the stage of disintegration of three micronuclei in each conjugation.

Autogamy

W.F. Diller (1936) described a process of nuclear reorganization in *P. aurelia*, resembling conjugation, but taking place within a single individual. He called it **autogamy** or **self-conjugation**.

[I] Process of Autogamy

During autogamy in P. *aurelia*, the 2 diploid micronuclei divide by meiosis to form eight haploid daughter nuclei. Seven of them disintegrate, while the remaining haploid micronucleus undergoes a mitotic division forming 2 gamete nuclei. Meanwhile, the macronucleus grows into an irregular skein-like mass, which breaks into pieces later to be absorbed in the cytoplasm. The two gamete nuclei enter a protoplasmic cone temporarily formed near cell mouth and then fuse together to form a completely homozygous diploid zygote nucleus or **synkaryon**. This divides twice to yield 4 nuclei, 2 of which become macronuclei and 2 micronuclei. The cell body and the micronuclei then divide to form two daughter individuals, each with a new macronucleus and 2 micronuclei. Autogamy rejuvenates *Paramecium*.

[II] Genetic Consequences of Autogamy

If autogamy takes place in a *Paramecium* heterozygous for a dominant gene (Aa), the resulting progeny will depend upon the survival of the gene A or a. If the gene A survives, it will lead to AA individuals or vice versa. Thus, autogamy always results in homozygosity.

Cytogamy

In 1940, **R. Wichterman** reported, in *P. caudatum*, a sexual process without nuclear exchange, termed **cytogamy**. The process resembles **conjugation** in that two small paramecia (200μ long) temporarily fuse by their oral surfaces. The early nuclear divisions are also similar to those of conjugation; but there is no nuclear exchange between the individuals (**cytogamonts**). But, two haploid **gamete nuclei** in each individual are said to fuse to form a **synkaryon**, as in **autogamy**. The process is completed in about 13 hours.

Endomixis

Endomixis (Gr., **endon**, within + **mixis**, mingling) is an interesting phenomenon involving a total internal nuclear reorganization within a single individual in a culture of a pedigreed race of *Paramecium*, taking place in the absence of conjugation. **Woodruff** and **Erdmann**, in 1914, first of all reported endomixis in the bimicronucleate species, *P. aurelia*, occurring

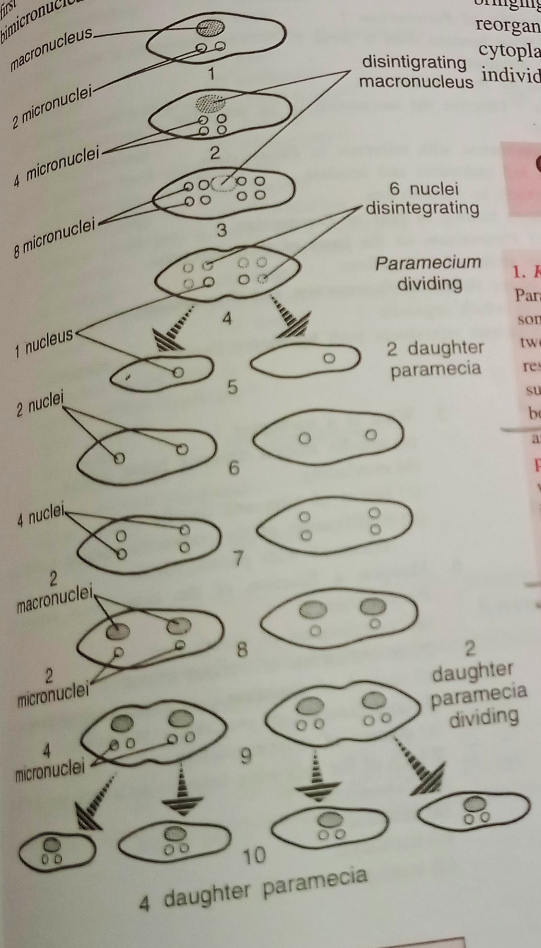


Fig. 20. *Paramecium.* Stages in endomixis.

periodically at regular intervals of about 30 days. The whole process may be summarized as follows :

The vegetative **macronucleus** degenerates and disappears, while the **micronuclei** divide twice by mitosis forming 8 **daughter nuclei** of which 6 degenerate. At this stage *Paramecium* also divides, and each daughter paramecia receive one micronucleus. This micronucleus divides twice forming 4 nuclei, 2 of which become macronuclei and 2 micronuclei, in each individual.

The micronuclei again divide with the binary fission of *Paramecium* into two daughters, each getting one macronucleus and 2 micronuclei. Thus, four daughters are produced from a single parent bringing about an intracellular nuclear reorganization and readjustment between the cytoplasm and the nuclear apparatus in each individual.

CYTOPLASMIC PARTICLES IN *PARAMECIUM*

1. ***Kappa* particles.** At the time of mixing of two races of Paramecia for conjugation, **T.H. Sànneborn** found that sometimes one race survives and the other dies out. These two races have been designated as **killer** and **sensitive**, respectively. It was found out that in the individuals which survive (**killers**) occur special self-replicating cytoplasmic bodies containing DNA, called **kappa particles**. These are associated with the production of a killing substance, **paramecin**. This substance diffuses out into the surrounding water and causes the death of the sensitive (kappa free) individuals. In the course of studies about the kappa particles, it was found that a dominant gene (K) in nucleus is necessary for kappa to exist, multiply and produce paramecin. Kappa particles provide an example of cytoplasmic inheritance. They are transmitted directly by cytoplasmic genes (plasmagenes) from cytoplasm of parent cell to the daughter cells, and not by nuclear genes as in ordinary heredity.

2. ***Pi* particles.** These are mutant forms of kappa particles. They do not release any toxic substance meant for killing those which are without such particles, that is, the sensitives.

3. ***Mu* particles.** These particles are also killers and kill the mate without such particles, during conjugation.

4. ***Lambda* particles.** These particles are borne by killer paramecia and cause the sensitive paramecia to lyse or disintegrate.