# Movements in rotifers: correlations of ultrastructure and behavior

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## Abstract

In this review, original observations on rotifer cilia and muscles are used to explain the mechanism of their movements. The following topics are discussed: (1) cilia and infraciliature of the cingulum, (2) muscle insertions into the infraciliature, (3) innervation of the muscles and non-innervation of the ciliated cells, (4) recent data on the location of gap junctions which link some muscles, (5) cilia and infraciliature of the cirri of the pseudotrochus of *Brachionus calyciflorus*, and the insertion of muscles onto their infraciliature, (6) the relation between muscles and integument in the foot of *Philodina roseola*, (7) the relation between muscles and integument in the joint present at the base of the tail of *Trichocerca rattus*, and (8) hypotheses on the first articulated (muscle-integument) movements which exist alongside ciliary motion in certain rotifers.

Neuro-ethological summaries, based on data of the effectors and on observations on behavior and the sensitivity of rotifer sensory receptors (reviewed in Clément *et al.*, 1983), are attempted. A more complete analysis is provided in the following instances: (1) swimming and the mechanism of turning, (2) retraction of the rotatory apparatus, (3) feeding behavior in *Trichocerca rattus* involving the succession swimming, crawling and nutrient intake.

Other behavioral sequences, for which the ethological and ultrastructural data are incomplete, are presented and briefly discussed.

Lastly, the argument is put forward that rotifers constitute an exceptional model for comparative, neuro-ethological studies which may enable us to better understand the mechanisms of evolution. Carrying out detailed observations, aimed at understanding how certain movements occur, should enable us to gain insights into the question of why they exist.

## 1. Introduction

To the naked eye a rotifer, if it can be seen at all, appears to be a fixed point in suspension that is moved about only by the water currents. One is led to ask if it has any movements of its own and what their utility might be, besides keeping the animal afloat as it is being carried along by the currents. However, as soon as one begins to observe the behavior of rotifers more precisely, the efficiency of their movements becomes evident, e.g. the positive phototaxis of certain planktonic species.

How and why do rotifers move? The question of 'how' is the easier of the two to take up, and the bulk of this review paper attempts to answer it. I will do that by establishing correlations between the motor sequences of behaviors (the movements) and their morphological basis, analyzed at the ultrastructural level. The question of 'why' is more complex and more philosophical, and it depends upon the point of view of the observer. Further, it is difficult to avoid anthropomorphisms when considering this question.

The question of 'why' deals with adaptation and,

consequently, with the various geneses of a rotifer: its phylogenesis, its ontogenesis, its individual life history and that of its parents and grandparents. All of this occurs within one or several precise ecosystems which change in the course of these geneses.

The movements of a rotifer depend upon its perception of the environment (cf. Clément et al., [1983]: a review of rotifer sensory perception). However, sensory perception varies with the movements of the animal and, what is more, certain perceptions are only possible as a result of movement [Campan & Lambin, 1984; Clément, 1984]. Therefore, it is somewhat artificial to separate movement (motor sequence) from sensory perception in the analysis of behavior. After I have described the ultrastructure of the motor basis of a behavior, I shall place it in a larger context which includes the sensory receptors, the nervous system, etc.

Neuro-ethological analysis is one of the two essential points of this work [see also Clément & Amsellem, in press]. Rotifers provide a particularly suitable model in this respect since they are characterized by a nervous system consisting of less than 200 neurons, very specialized sensory receptors and muscular effectors made up of only a few cells, eutely, and diploid parthenogenesis.

The second point is etho-ecological and it is also directed at the mechanisms of evolution. Behavior seems to act as a motive force in evolution [Waddington, 1974; Piaget, 1976]. A comparative study of the behavior of various rotifer species and clones (and of the ultrastructure of the morphological basis of the same) might interest ecologists as well as those who are interested in the evolution of rotifers.

## 2. Swimming (Plates I, II, IVa; Figs. 1 and 2)

Swimming characteristics of rotifers vary with different environmental conditions [Clément et al., 1983]. The present review describes more precisely the cilia of the cingulum and the muscles inserted onto their infraciliature. I then discuss data regarding the effectors in light of observations on sensory receptors already published [Clément, 1977a, 1977b, 1984a; Clément et al., 1983] in order to pro-

vide some hypotheses concerning the mechanisms of swimming.

### a) Coordination of ciliary beat in the cingulum

Coordination of ciliary beat makes the cingulum looks like wheels at the anterior end of rotifers. Several workers have taken advantage of this feature and have measured the frequency of ciliary beat using a stroboscope [Lansing & Lamy, 1961a; Luciani, 1982; Kutikova, 1983].

The mechanism by which ciliary coordination is achieved is not known, but we may infer that the infrastructure of the cilia of the cingulum plays a major role in it, for four reasons. 1) There are always horizontal connections between the bases of adjacent cilia in the cingulum (Plates I and II). 2) The ciliary rootlets are always associated with mitochondria and the endoplasmic reticulum [Plates I and II; Clément, 1977a; Luciani, 1982; Cornillac, 1982]. 3) Horizontal components of the infra-ciliature, present either at the apex of the cell (Plate IIb) or below the apex (Plate IIa), are inserted onto epithelio-epithelial desmosomes which assure the mechanical coherence of the infraciliature of several adjacent cells in the cingulum (Plate IIa). 4) Elsewhere, the epithelial cells of the cingulum are coupled by gap junctions (P. Clément, J. Amsellem and E. Wurdak, unpublished observations on Trichocerca, Philodina, and Asplanchna; Cornillac, [1982], and Luciani, [1982] on Brachionus).

The cilia of the cingulum show all the properties associated with motile cilia [Lansing & Lamy, 1961a; Clément, 1977a, b; Plates I & II]. Their movements are coordinated, without a doubt, by the infraciliature and by the plasma membrane which is also the membrane investing the cilia and which is in close contact with the axoneme (the tubulin-dynein complex responsible for ciliary movement) at the base of each cilium.

### b) Mechanism of turning in Brachionus and Asplanchna

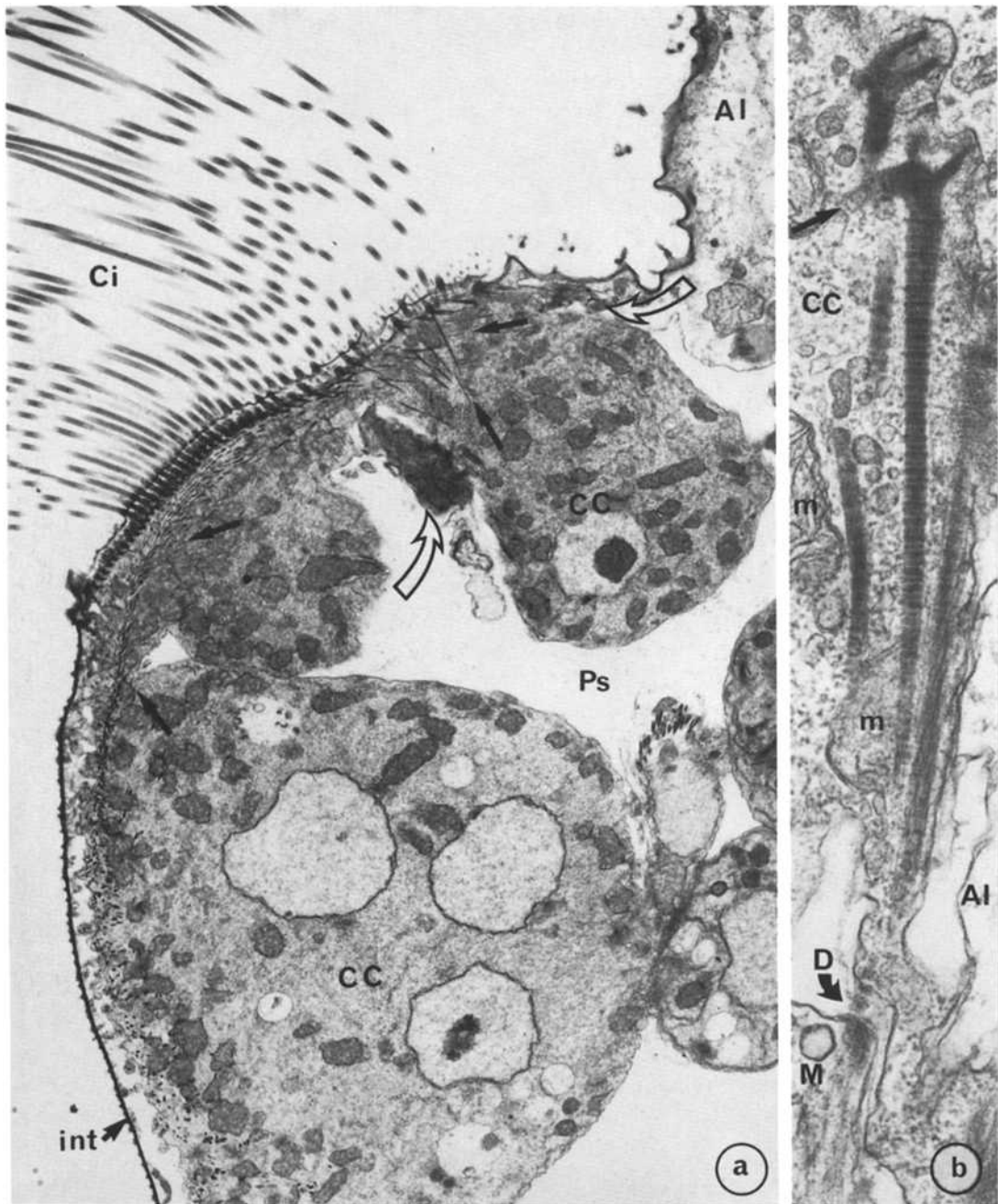Plates I and II show that some muscles (cingulum

*Plate I.* The cingulum of *Asplanchna brightwelli*

a. ×4300. Section through the ciliated cells of the cingulum (CC) containing several nuclei, glycogen particles, and mitochondria which are especially numerous near the ciliary rootlets. The rootlets bifurcate at the base of each cilium (solid arrows). The tip of each of these branches inserts onto a muscle (large open arrows). (AI — apical integument; Ci — cilia of cingulum; int — syncytial integument of trunk; Ps — pseudocoel).

b. ×42000. Details of the insertion of a ciliary rootlet onto a musculo-epithelial desmosome (D). The cingulum cell (CC) is very *close to the apical integument (AI). (M = muscle ending). Note the classical striation of the ciliary rootlets and their association with mitochondria (m) and endoplasmic reticulum vesicles.* Electron micrograph courtesy of Wurdak and Clément (unpublished).
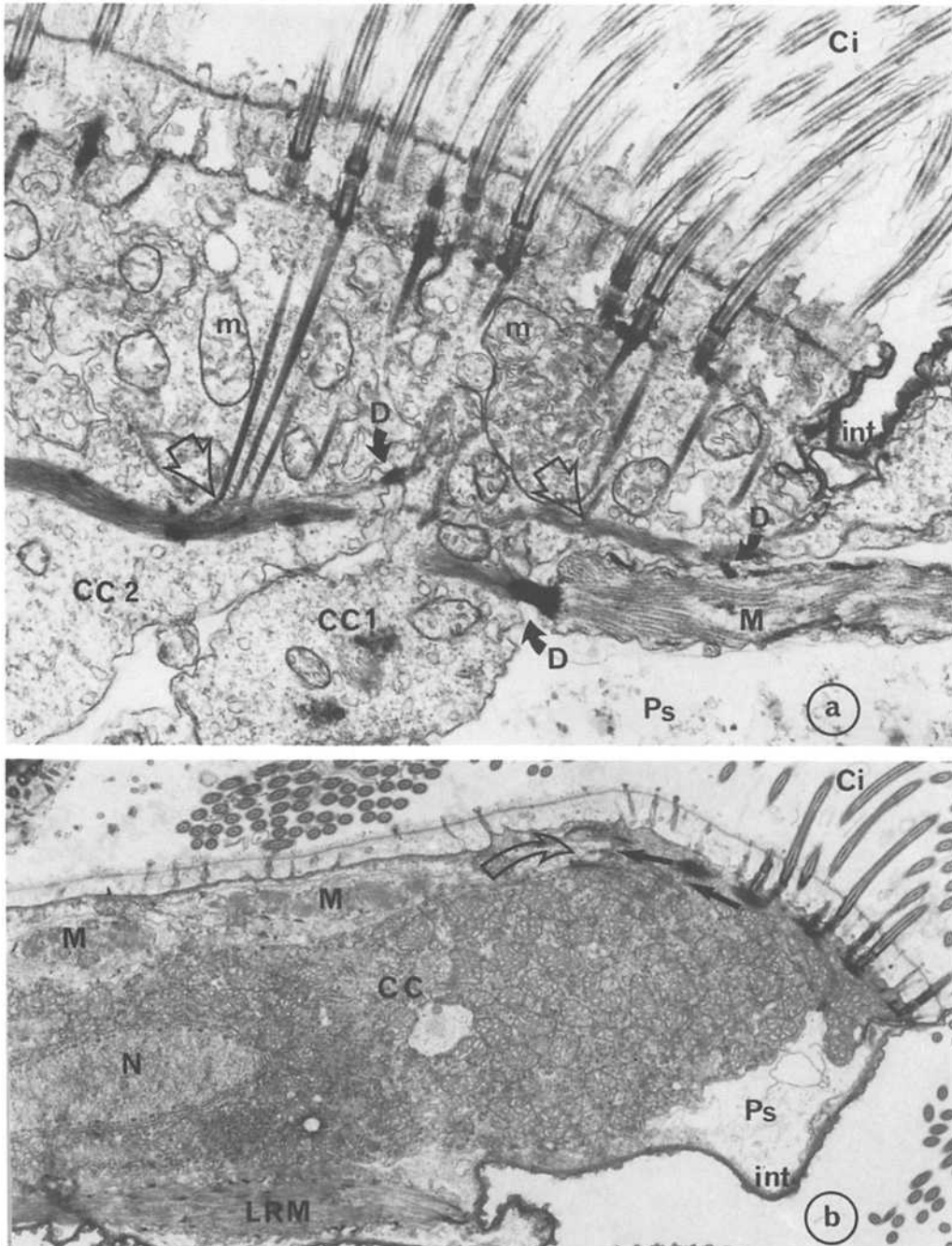
*Plate II.* The cingulum of *Brachionus*

a. ×18000. *Brachionus plicatilis.* The striated, vertical ciliary rootlets are inserted (empty, large arrows) onto the horizontal bundles of filaments which are, in turn, inserted onto desmosomes (D). Cilia borne by different cingulum cells (CC$_1$ CC$_2$) are connected to the cingulum muscle (M) in this manner. (m = mitochondrion; Ps = pseudocoel).

b. ×7800. *Brachionus calyciflorus.* In this species the striated ciliary rootlets lie close to the surface of the cell and extend horizontally, i.e. parallel to the surface (solid arrows). They attach to muscle ending (M) by means of a musculo-epithelial desmosome (large empty arrow). One of the lateral retractor muscles (LRM), cut axially, inserts onto the ventral integument (int). N = nucleus of the cingulum cell (CC). Electron micrograph courtesy of Amsellem, Clément, Cornillac and Luciani (Figure *11a* from Cornillac, 1982 & Luciani, 1982).
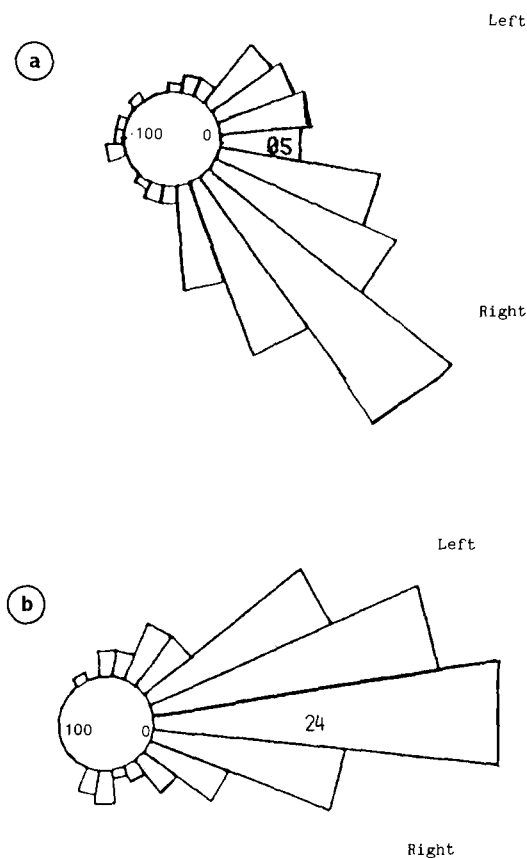
Left

Right

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muscles) have their insertions on the rootlets of the cingulum. The insertion of muscles onto ciliary rootlets have already been described, but it has not been mentioned whether the rootlets belong to the cingulum or the pseudotrochus [Clément & Fouillet, 1970; Clément, 1977a, b]. Other authors [Lansing & Lamy, 1961a, b; Scholtyseck & Danneel, 1962] have described the structure of ciliary rootlets, but they did not observe these musculo-epithelial desmosomes. In *Trichocerca rattus* there is a pair of cingulum muscles (Fig. 2): each of them passes through a nerve ganglion and it is richly innervated. Several synaptic buttons coming from different nerve endings have been observed [Clément, 1977a, b]. Two of these synaptic buttons are shown on Plate IVa; they enclose synaptic vesicles
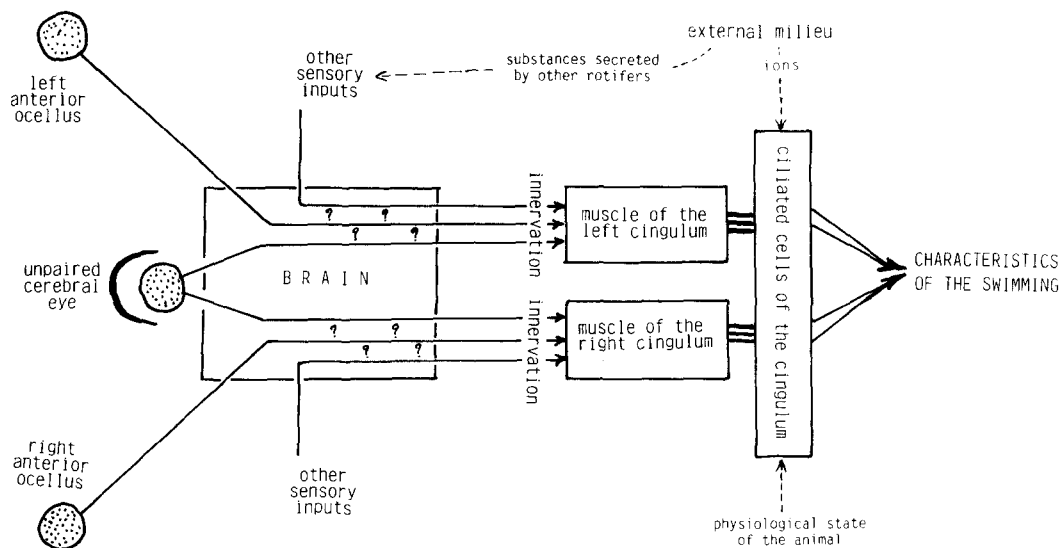


*Fig. 2.* Neuro-ethological schematic diagram of the regulation of swimming in response to illumination in a rotifer. Hypothetical interactions among sensory inputs in each half of the brain (left and right) are indicated by question marks. The three lines which connect the muscle to the cells of the cingulum represent musculo-epithelial desmosomes joined to the infraciliature of the cilia of the cingulum that are responsible for swimming [From Clément & Amsellem, in press].

which are similar in appearance to vesicles containing acetylcholine. However, other nerve fibers contain different vesicles [Villeneuve & Clément, 1971; Clément, 1977a, b].

I have never observed a direct innervation of cells making up the cingulum in any species (including *T. rattus* whose structure is quite well known). Therefore, one must conclude that the cingulum muscles and the infraciliature serve as intermediaries in the neural control of swimming.

Observations of swimming behavior in *Brachionus* and *Asplanchna*, carried out with a recently refined automatic tracking device [Coulon *et al.*, 1983, 1984; Clément *et al.*, 1984, 1985], have demonstrated a consistent, positive correlation between amplitude of turning and swimming speed. Swimming speeds decrease as the animal turns: the wider the angle of turning, the more marked the decrease in speed. Therefore, turning seems to be the result of the slowing down of either the right (right-hand turn) or the left (left-hand turn) half of the cingulum. This interpretation is supported by observations made with the aid of a stroboscope which show differences in ciliary beat between the right and the left half of the cingulum. It is also supported by observations on *Notomata copeus*. This animal is able to retract its two auricles independently of each other thereby illustrating the autonomous action of the different cingulum muscles.

### c) Helicoidal swimming

The swimming pattern of most rotifers is helicoidal. In *Brachionus* and *Trichocerca*, for example, the cingulum muscle is inserted onto the infraciliature which is arranged in a horizontal plane (i.e., perpendicular to the base of the cilia). This plane is apical in *Brachionus calyciflorus* (Plate IIb). It is made up of fairly voluminous, horizontal, striated ciliary rootlets that insert onto a musculo-epithelial or epithelio-epithelial (integument or anterior ciliated cells) desmosome [Clément, 1977a, b; Plate IIb]. This plane is situated at greater depth in the epithelial cells of *Brachionus plicatilis*. In this species it is composed of bundles of actin-like

microfilaments, that also insert onto desmosomes (Plate IIa) located either between two epithelial cells or between an epithelial cell and the cingulum muscle. In this arrangement, the cingulum muscle controls the ciliary rootlets of the entire cingulum by means of a single zone of insertion.

Muscular control is, at the same time, unilateral. This accounts for the fact that in *Brachionus*, the mean turning angle lies around 45° and it is to the right (Fig. 1). Almost all turns are toward the right; very rarely do we observe a left-hand turn. Therefore, we are probably dealing with turns where the animal is in a ventral position in its helicoidal swimming path.

### d) Non-helicoidal swimming (Asplanchna)

Ciliary rootlets in *Asplanchna* differ from those of *Brachionus* (Plate I). There is no horizontal plane perpendicular to the base of the cilia. Instead the base of each cilium bifurcates and extends into the cytoplasm as two ciliary rootlets which form an inverted V. A muscle inserts onto each extremity of the V. Therefore, each cilium is under control of two opposing muscles (Plate Ia, b). The resulting ciliary beat has two characteristics: 1) The pattern of swimming is not helicoidal. 2) A frequency histogram of turning angles in *Asplanchna* (Fig. 1) shows a distribution around 0°. There is no systematic tendency to turn toward the riht and the largest class of turning angles is in the −7.5° to +7.5° range. Outside of this range, frequency of turning angles diminishes as their amplitude increases.

### e) Factors influencing swimming path

One of the objectives of my research and that of colleagues has been to identify external stimuli that bring about a change in swimming path [cf. Clément *et al.*, 1983]. Luciani *et al.* [1983] have shown that swimming characteristics in *B. plicatilis* vary with age. Luciani [1982] has noted other age-related variations in the same species (e.g., decrease in ciliary beat, loss of nutrient reserves in cells of the

cingulum, decrease in ATP content of whole animals, an increase followed by a decrease in size and number of mitochondria present in cells of the cingulum, lysosome accumulation, and progressive vesiculation of cells of the cingulum).

Luciani *et al.* [1983] also have shown that the presence of calcium in the medium can influence some of these age-related changes in swimming behavior. These results are more difficult to interpret; for example, is this a direct influence of calcium on the cilia, and/or the influence of calcium present within the animal, notably within the cells of the cingulum? Experimental results on the influence of inherited calcium on longevity support the second hypothesis [C. E. King, pers. comm.].

Wallace [1980] likewise has demonstrated that swimming speed varies with age in the larvae of the sessile rotifer *Ptygura beauchampi*. In this case, however, the animal is still young and no degradative mechanisms are likely to be involved. Perhaps this decrease in swimming speed is the result of depleted energy reserves or a developmental effect on swimming behavior.

### f) Photokinesis: Acceleration and deceleration of ciliary beat

Viaud [1943c] studied ciliary beat in a number of phototaxic rotifers with the aid of a stroboscope. He showed that variations in illumination have no effect on the rhythm of ciliary beat, but they do have an effect on its amplitude. This effect probably explains changes in swimming speed first noted by Viaud [1943a, b] and later by Clément [1977a], Cornillac [1982] and Clément & Wurdak [1984] that follow changes in the intensity or quality of light. However, recent results differ from those of Viaud [1943a, b] in certain respects, particularly in the influence of wavelength, but they also confirm the existence of a photokinesis. It would be desirable to repeat Viaud's stroboscopic investigations using isoquantic, monochromatic light.

One of the aims of this research would be to identify the photoreceptor (eye or ocellus: Clément *et al.* [1983]; Clément & Wurdak, [1984]) responsible for photokinesis, another would be to deter-mine whether the same sensory-motor pathways are involved in photokinesis as in phototaxis (Fig. 2). Generally speaking, acceleration or deceleration of cilia of the cingulum following stimulation (light, currents etc.) might be the result of a slight contraction of the two cingulum muscles simultaneously (Figs. 2 and 3).

### g) Synthesis (Fig. 2)

Figure 2 summarizes the observations discussed above. The right cingulum muscle is innervated independently of the left cingulum muscle. The sinuosity [Bovet, 1984] of swimming depends on the autonomy of right and left halves of the cingulum.

Figure 2 also summarizes information concerning the brain and the sensory receptors. The brain shows a clear bilateral symmetry, which had been observed with both the light [Nachtwey, 1925; Remane, 1929–32] and the electron [Ware, 1971; Clément, 1977a, b] microscope.

The sensory receptors themselves are also symmetrical. There are paired organs, unpaired organs consisting of paired neurons (e.g., dorsal antenna), and unpaired organs consisting of unpaired neurons that send two axons, one to each half of the brain (cerebral eye of *Asplanchna brightwelli* and *Brachionus calyciflorus*) [Clément *et al.*, 1983; Clément & Wurdak, 1984; Clément, 1984a]. There seem to be, therefore, nerve tracts that originate at sensory receptors and course independently through the right or left half of the animal all the way to the right or left cingulum muscle.

The central point of neuro-ethological analysis currently lies in the nervous network. In this regard several important questions need to be addressed. (1) Is there interaction among different sensory modalities at the level of the brain? (2) Are some modalities pre-eminent over others? (3) Why is there multiple innervation of each muscle of the cingulum? The study of behavior (different sensory influences on swimming) combined with studies of neuro-anatomy may yield answers to these questions.
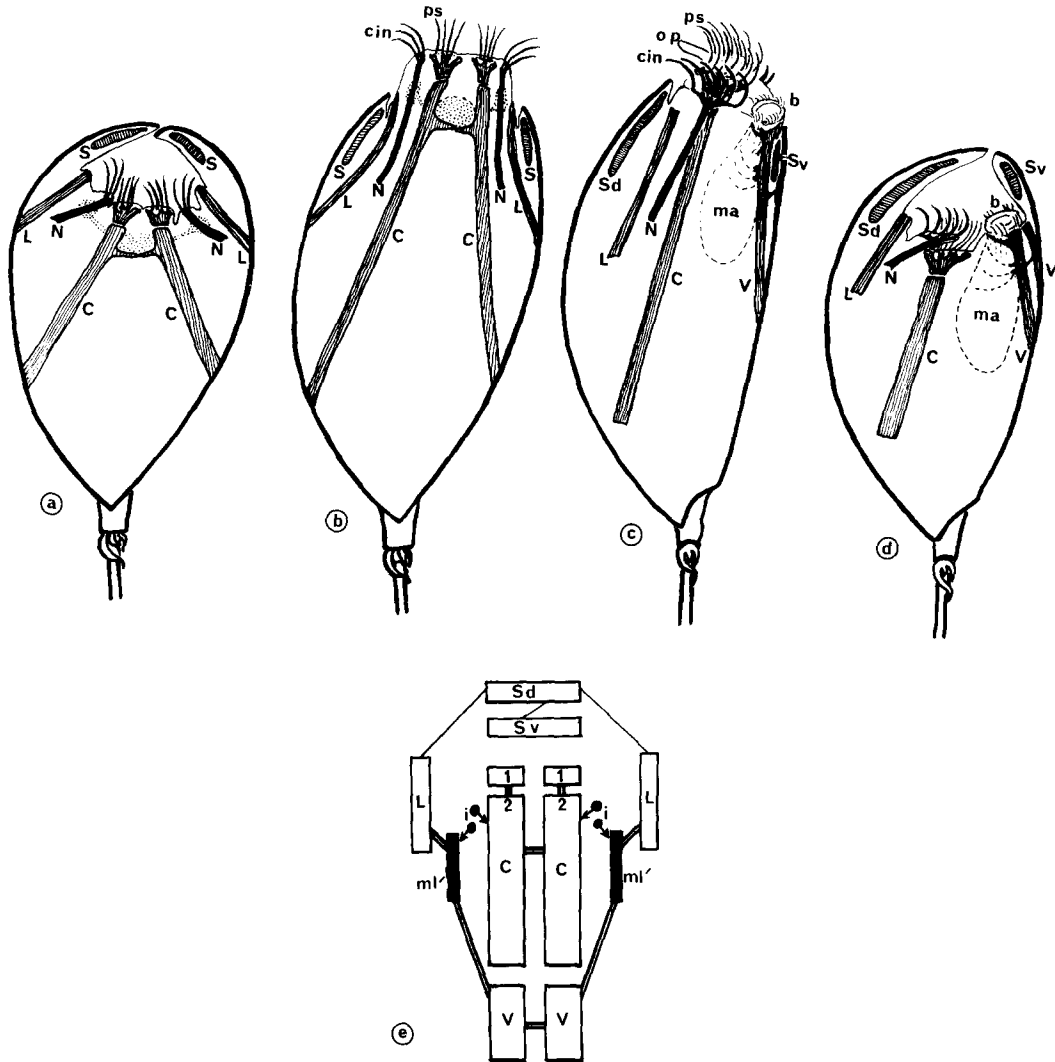
*Fig. 3.* A diagram of the muscles involved in withdrawal of the corona in *Trichocerca rattus*. a and b: dorsal views, the brain is stippled; c and d: lateral views; b and c: relaxed animals; a and d: contracted animals; e: diagram of the gap junctions present between the muscles. A double line between 2 cells indicates that the junctions have been observed; a single line indicates that such junctions are very likely to be present (the plasma membranes are in close contact) but have not been actually observed (the angle of sectioning was unfavorable). Abbreviations: b = mouth and ciliated buccal field; C = central retractor muscles (cells 1 and 2); cin = cilia of the cingulum; L = lateral retractor muscles; ma = mastax; N = muscles of the cingulum; op = palpar organ; ps = cilia of the pseudotrochus; S = anterior sphincter made up of 2 circular muscles, one dorsal (Sd) and one ventral (Sv). [From Clément and Amsellem, in press].

## 3. Retraction of the rotatory apparatus

Retraction of the rotatory apparatus is a widespread and frequent behavior among rotifers, which is especially spectacular in bdelloids and sessile rotifers. The only exceptions to this are those species that possess body wall expansions (spines or paddles) at the anterior boundary of the integument of the trunk (cf. part 4 of this review).

The majority of rotifers withdraw the rotatory apparatus into the interior of the trunk at the slightest provocation (e.g., touch, chemicals, vibration, etc.). Certain stimuli such as coming into contact with an obstacle, trigger only a slight retrac-

tion, while others cause a more complete retraction with a final closure of the opening which separates the rotatory apparatus from the external medium. The trunk integument of the anterior end of the animal consequently serves as a protective covering over the rotatory apparatus and its numerous sensory receptors.

### a) Muscular insertions

Jacqueline Amsellem and I have studied the ultrastructure of the muscles involved in this behavior using serial sections of *Trichocerca rattus* [Amsellem & Clément, 1977 and unpublished data; Clément & Amsellem, in press; Amsellem, 1985].

The only muscles concerned with this movement are 3 pairs of longitudinal muscles (central, ventral and lateral retractors) and one pair of anterior circular muscles (one member of the pair being dorsal and the other ventral) (Fig. 3). All the retractors are inserted onto the trunk integument at their base. For an initial explanation of their function their apical insertions must be examined in detail. (1) The central retractors have their insertions under the central region of the apical field and the pseudotrochus. (2) The ventral retractors insert into the peripheral muscles of the mastax, the mouth, and the ciliated cells surrounding the mouth. (3) The lateral retractors have their insertions on the soft integument anterior to the trunk.

The anterior circular muscles are inserted strictly onto the integument of the trunk at various points corresponding to the Z lines. Plate Vc shows the ultrastructure of one of these insertions in detail. A desmosomal plate, consisting of dense bodies which are the sites of attachment of the thin (actin) myofilaments exists on the muscle side of the insertion. On the epithelial side there are only filaments which join the intracytoplasmic skeletal lamina to the desmosome. Such muscle-integument junctions have already been described in the Rotifera [Koehler, 1965; Clément, 1969, 1977a, b, 1985; Storch & Welsh, 1969]. A precise knowledge of all the muscular insertions permits us to have an understanding of the respective functions of all 3 pairs of

retractors and that of the 2 anterior circular muscles which form an incomplete sphincter.

### b) Correlations between muscle cytology and function

All the muscles in question, except the central retractors, are unicellular. The central retractors are bicellular. Therefore, they represent an interesting system for a detailed ultrastructural study (quantitative cytology included) of the entire muscle. These cytological results and other observations, such as, the extent of contraction of these muscles following fixation [Amsellem & Clément, 1977], the position of the retracted organs, and the dynamics of the process of retraction, are good indicators of their physiological characteristics. Data that have been obtained are too voluminous to be presented or even summarized here. They lead to the following conclusions [Amsellem, 1985]. 1) The central retractors contract very rapidly over a long distance. They are the longest and they are often found in a supercontracted state. These muscles are likely to be the least enduring (i.e., the contraction is of short duration). 2) The shorter ventral retractors seem to contract somewhat more slowly and to be a little more enduring. 3) The lateral retractors and the anterior circular muscles have a definitely slower and more protracted contraction.

From this information it is possible to reconstruct the sequence of events which lead to the contraction of the corona. First the apical part of the rotatory apparatus is pulled into the trunk by the central retractors. The buccal zone and the mastax are pulled in at about the same time, but somewhat slower and not as far, by the contraction of the ventral retractors. Finally, the lateral retractors and the anterior circular muscles bring about a prolonged retraction.

Termination of retraction is not due to contraction of antagonistic muscles, but to the progressive relaxation of the same 4 pairs of muscles which initiated it (especially the most enduring ones: lateral retractors and anterior circular muscles). The internal pressure is then sufficient for the reappearance of the corona.
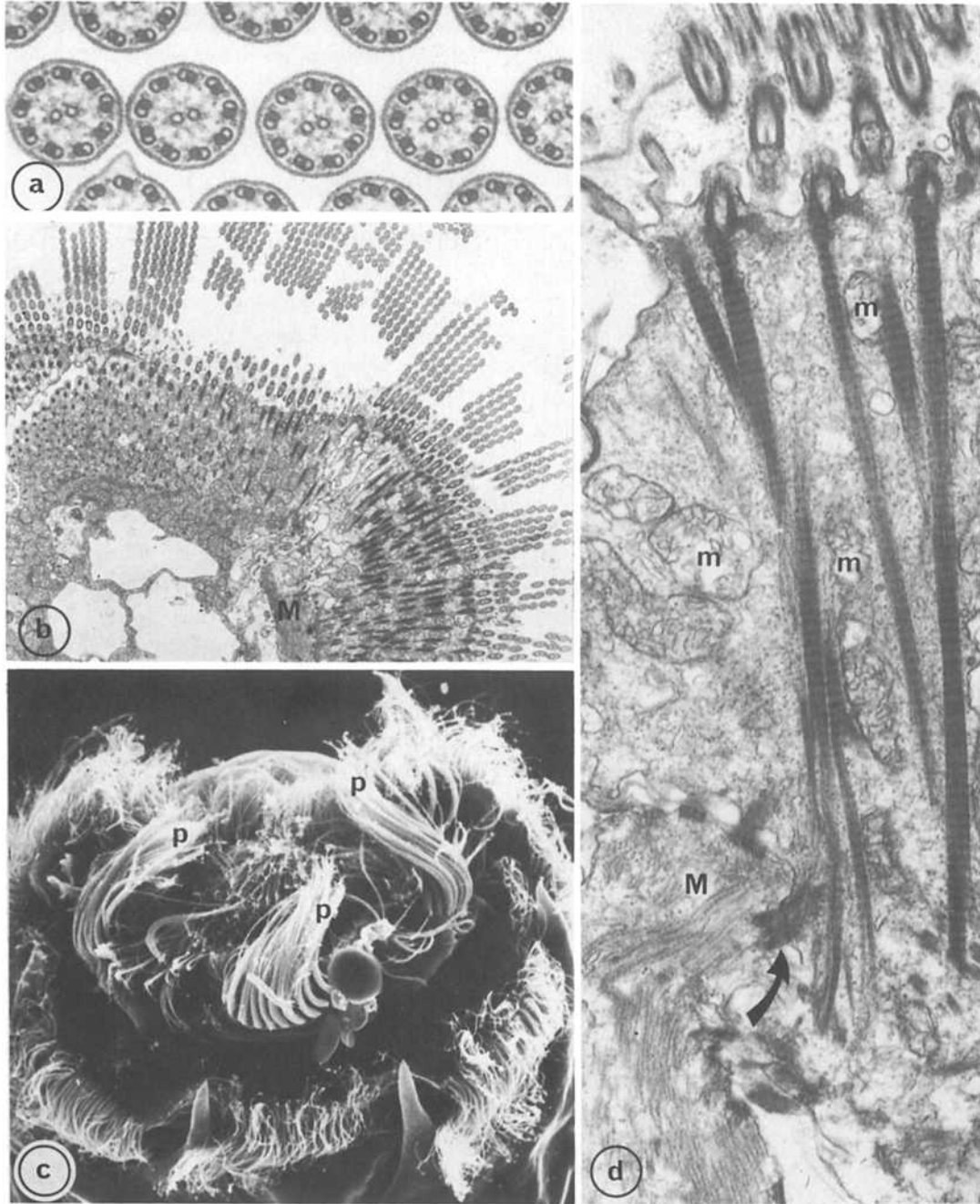
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*Plate III.* The pseudotrochus of *Brachionus calyciflorus*

a. ×80000. Transverse section through the cilia of one of the cirri of the pseudotrochus. The cilia are arranged in rows and they beat in unison perpendicularly to the plane of the row, which is determined by the position of the central tubules.

b. ×4000. Transverse section through one of the 3 bundles of cirri. Each cirrus consists of 4−8 rows of cilia in close registry.

c. ×800. Dorso-apical view of the rotatory apparatus under the scanning electron microscope. The pseudotrochus (P) consists of 3 bundles of cirri in the inner corona.

d. ×24000. The striated rootlets of the cilia of the pseudotrochus are inserted onto musculo-epithelial desmosomes (arrow). Mitochondria (m) lie in close proximity to the rootlets. M = muscle. Electron micrograph courtesy of Amsellem, Cornillac & Clément (unpublished).
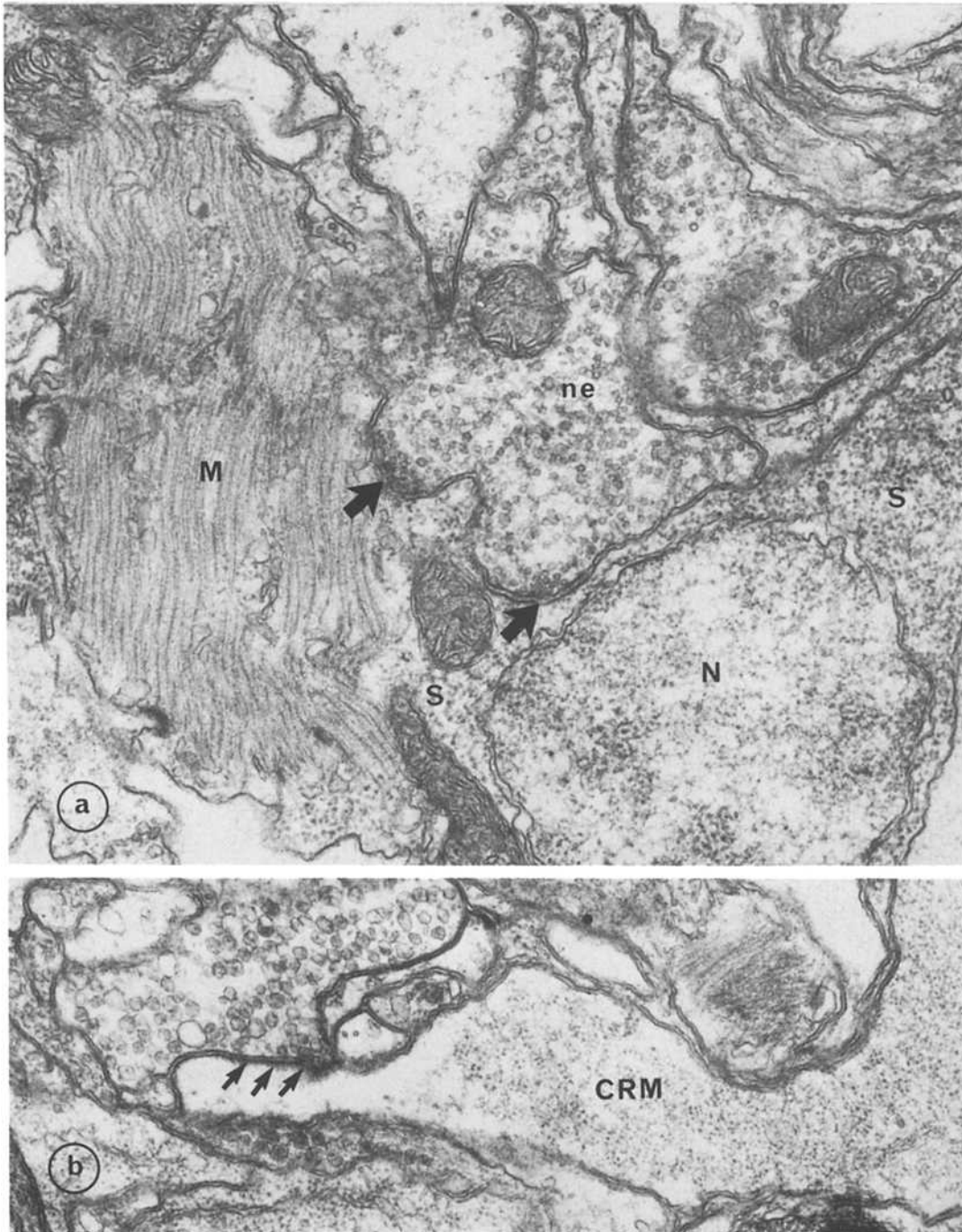
*Plate IV.*

a. Innervation of the cingulum muscle in *Trichocerca rattus*. ×27000. The two synaptic boutons (arrows) in this section come from one nerve ending (ne) and innervate the muscle near its nucleus (N). (M = myoplasm; S = sarcoplasm).

b. Innervation of the central retractor muscle (CRM). ×32000. The arrows point to the post-synaptic membrane at the upper part of the sarcoplasmic extension (CRM) which penetrates into the brain. Note the size of the vesicles present in different nerve fibers. The nerve fiber which makes synaptic contact with the muscle contains larger vesicles than those seen in the previous micrograph. Electron micrographs by Amsellem and Clément (from Clément, 1977a).

## c) Innervation and gap junctions (Plate IVb; Plate Va; Fig. 2e; Fig. 4)

An ultrastructural study, carried out with the aid of serial sections, allowed Clément & Amsellem [1985] to localize the innervation of these muscles and the gap junctions which unite them. Some important observations of that study are shown on Figs. 2e and 4. They may be summarized as follows. 1) The two central retractors are clearly coupled (Plate Va), as are the ventral retractors and the 2 cells making up each central retractor. 2) Only the central retractors are innervated, each by nerve fibers which extends into the brain and receive several synaptic endings (Plate IVb). 3) None of the other 3 pairs of muscles involved in the retraction of the corona seems to be innervated. However, they are connected via gap junctions to each other and to a small lateral muscle (ml'), which is innervated. Hence there is an electrical coupling among them.

Two important conclusions regarding the process of retraction may be drawn from these observations. 1) The left and right sides of an animal are always coupled in retraction, whereas during swimming they are not. 2) Two sets of muscular effectors act as a unit: the central retractors, on one hand, and the 3 other pairs of muscles, on the other.

## d) Synthesis (Fig. 4)

The diagrammatic summary of retraction (Fig. 4) differs from that of swimming (Fig. 2). There is always a total response (left and right sides acting in unison) even if the stimulus reaches only one of the two paired sensory receptors involved.

The two effector units are innervated laterally (left and right). This means that there is probably no communication between the two halves of the brain, at least as far as this behavior is concerned. Coupling is at the level of the muscular effectors. It is possible that initiation of this behavior (in response to a brief contact between the animal and an obstacle) corresponds to the beginning of the contraction of the central retractors. Only prolonged stimulation would bring about supercontraction of the central muscles and the contraction of all the other muscles involved in retraction. Synapses in the brain would be the basis for this sensory-motor integration. Unfortunately they have not been mapped yet.

## 4. Musculo-integumentary movements (Plates Vb, VI & VII)

Ciliary swimming is possible in rotifers because of their small size [Epp & Lewis, 1984]. However, certain species of the genera *Polyarthra* and *Filinia*
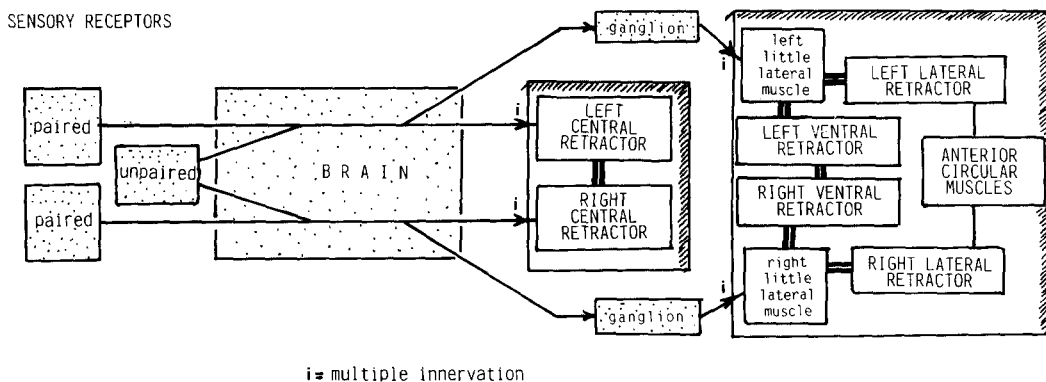


*Fig. 4.* Neuro-ethological schematic diagram of the withdrawal behavior in *Trichocerca rattus*.
    The stimulation may come from one side only; however, the response is always global. If only the central retractors respond, a slight retraction results; if all the muscles shown respond, total retraction follows. The connections between muscles are indicated by double lines (observed gap junctions) or single lines (probably gap junctions). [From Clément & Amsellem, in press].
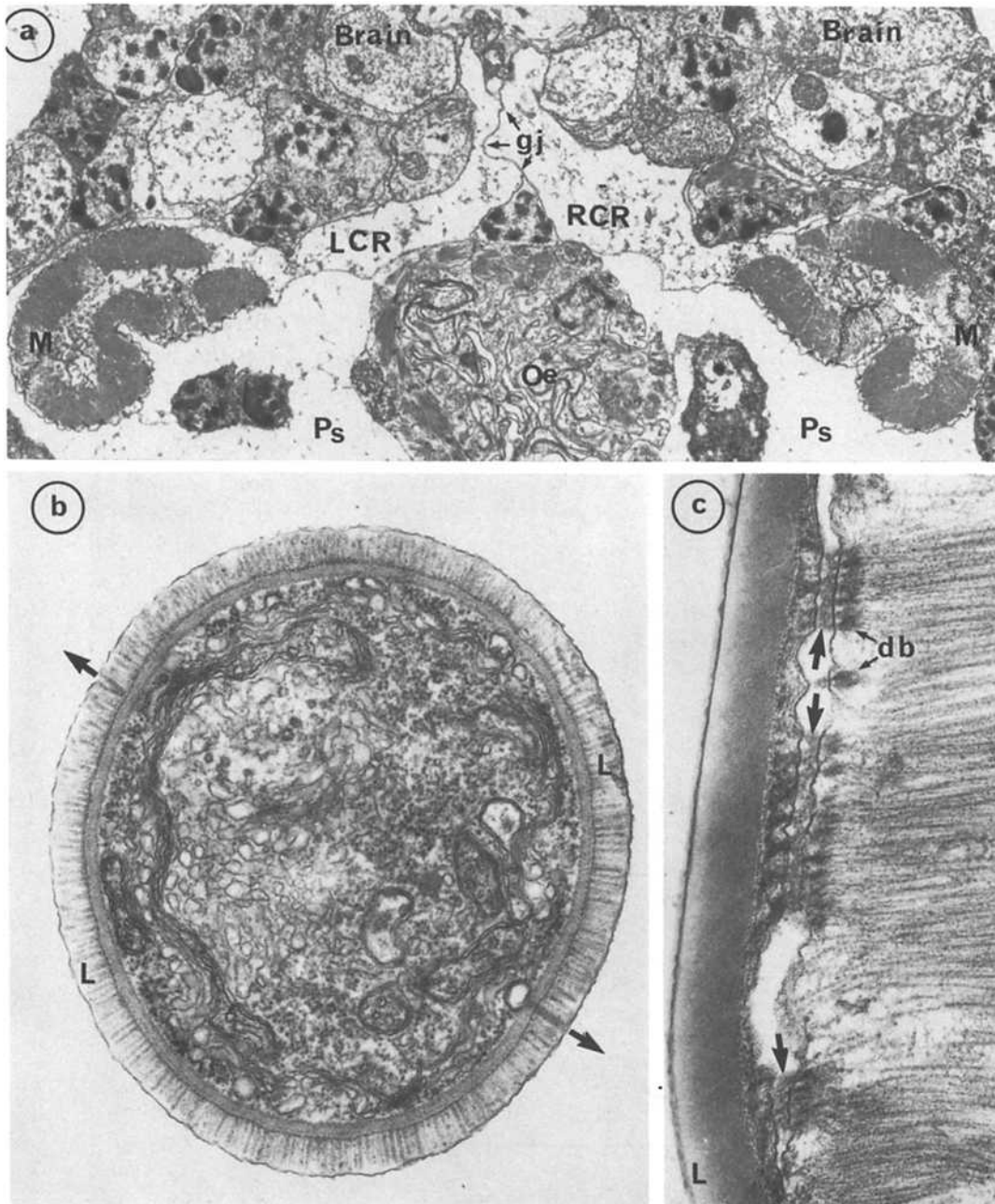
*Plate V.*

a. Gap junction (gj) between the sarcoplasm of the left central retractor (LCR) and the right central retractor (RCR) in a transverse section of *Trichocerca rattus*. ×7200 (Oe = esophagus, M = myoplasm, Ps = pseudocoel).

b. Spines in *Brachionus calyciflorus*. ×26500 − Transverse section of the posterolateral spine of a *Brachionus calyciflorus* female whose mother had been exposed to water in which *Asplanchna brightwelli* has been cultured. Note that the intracytoplasmic skeletal lamina (L) is quite thick and has the typical structure described in *Brachionus* [Clément, 1969]. There is no pseudocoel in the spine at this level. The cytoplasm of the integument contains a well developed Golgi apparatus, which participates in the processing of integumentary secretions (arrows).

c. Insertion of the anterior circular muscle on the integument of *Trichocerca rattus*. ×50000. Arrows point to the central stratum present in the intercellular space at the desmosomes. Note the dense bodies (db) in the muscle cell cytoplasm which are aligned next to the plasma membrane at the desmosome. Myofilaments of actin are inserted on these dense bodies. In the integumentary cytoplasm dense bundles of filaments join the plasma membrane to the intracytoplasmic skeletal lamina (L). Electron micrographs by Amsellem, Clément and Cornillac [*Va* from Amsellem, 1985].

*Plate VI.* Axial section through the foot of *Philodina roseola.* ×6600. Oblique, transverse, and axial sections of the pedal ducts show at least 2 kinds of secretions which are discharged into the external medium (Ext) [pe = external pedal secretions]. The pseudocoel (Ps) contains longitudinal sections through several paired muscles ($M_1$, $M_2$, $M_3$, $M_4$, $M_6$) which are inserted on the soft integument of the foot and its invaginations. A longitudinal skeletal muscle (SM), sectioned axially, is inserted on the more rigid integument of the trunk (arrow) at the upper left. Electron micrograph by Amsellem and Clément [unpublished].
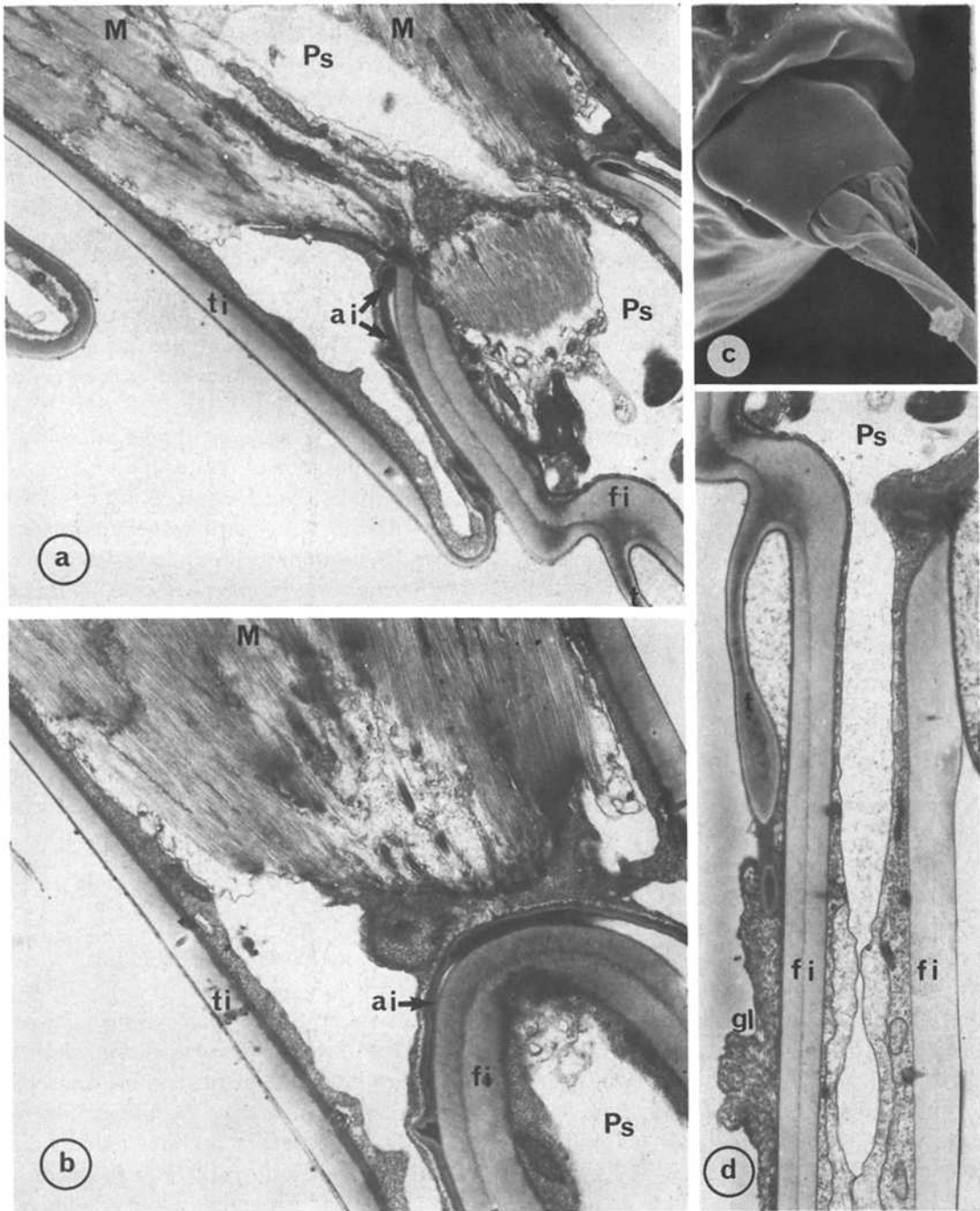
Plate VII. Axial sections through the foot of *Trichocerca rattus*.

a. ×18000 and b ×22000. The principal muscle fibers (M) are inserted at the junctions of the rigid foot integument (ti) and the soft articular integument (ai). The integument at the base of the tail (fi) is twice as thick as that of the trunk and of the base of the foot (ti). Ps = pseudocoel.

c. ×1750. Scanning electron micrograph of the posterior extremity of the trunk and the base of the foot which bears the tail and several small toes.

d. ×22000. Axial section through the base of the tail and one tow. The tip of the toe is embedded in the glue (gl) secreted by the pedal gland of *T. rattus*. The tail integument is (fi) twice as thick as that of the trunk and of the base of the foot. The pseudocoel reaches into the base of the tail. The tip of the tail is filled by the cytoplasm of the integument (ti = integument of the trunk and of the foot, fi = integument of the tail). Electron micrographs by Amsellem and Clément [VIIc from Clément, 1977a].

utilize, on occasion, another mode of locomotion to quickly move away from predators. This movement is a sudden jump and is accomplished by powerful muscles acting upon expansions of the integument which articulate with the trunk integument at their base. Gilbert [1985, 1987] has analyzed the jumps of *Polyarthra* through high speed cinematography. However, at present, we lack precise ultrastructural observations that would assist us in understanding the mechanism of these movements. Nonetheless, they have considerable adaptive significance, since they permit displacements which are very rapid in *Polyarthra*: 92 mm per second versus 0.7 mm per second in normal swimming [Gilbert, 1985].

However, we currently have some ultrastructural data that may help us in understanding the phylogenesis of these crude beginnings of articulated movements in rotifers. I will consider them briefly before I return to the mechanism of jumping movements in *Polyarthra* and *Filinia*.

### a) Retraction of the corona and foot

The beginning of an articulation is present in two areas, corona and foot. The rigid trunk integument lies next to the soft integument which connects the trunk to the rotatory apparatus. The lateral retractors are inserted directly onto the soft integument which folds inward (Plate IIb; Fig. 3).

Retraction of the foot, particularly in bdelloids (Plate VI), illustrates how articulated movements and telescoping structures are made possible by alternation of rigid and soft integument. Here again, various paired muscles of the foot are inserted on the soft articular integument at each telescoping zone (Plate VI).

### b) Movements of the tail of Trichocerca rattus (Plate VII)

These movements, though built on the same principle described above, show a more elaborate articulation. The tail is simply a very long cylinder made up of syncytial integumentary epithelium which has an intracytoplasmic, dense, skeletal lamina that is double the usual thickness. At its base the tail bears a ball and socket joint. Powerful foot muscles are inserted onto the soft part of the integument at several sites in the joint. This arrangement explains the possible movements of the tail of this rotifer.

### c) Spines of Brachionus calyciflorus (Plate Vb)

These spines appear only in the descendants of females that have been exposed to water in which their predator, *Asplanchna* has been cultured [de Beauchamp, 1952a, 1952b; Pourriot, 1964, 1974; Gilbert, 1967]. They are simple extensions of the syncytial integument (Plate Vb).

Analysis of filmed sequences [Clément & Pourriot, 1982] show that their spines articulate at their base. Upon coming into contact with an *Asplanchna* female, *B. calyciflorus* retracts its rotatory apparatus and, at the same time, extends its spines in a direction perpendicular to its body. It remains immobile in this manner until swimming is resumed at the end of coronal retraction. With the resumption of swimming, the spines are returned to their initial position, parallel to the trunk.
It is possible that the retraction of the rotatory apparatus causes spine movement by an increase of the internal pressure.

### d) Paddles of Polyarthra and Filinia

In contrast to the previous example, movement of these expansions of the integument does not accompany retraction of the corona, but replaces it. In this case, most or all of the longitudinal retractor muscles might have a modified anterior insertion that enables them to participate in the articulated motion. An ultrastructural study on *Filinia longiseta* which is currently in progress by Clément and Cornillac might bring us the answer to this question.

### 5. Crawling and feeding in rotifers which graze on filamentous algae (Figs. 5 & 6)

Feeding behavior of *Notommata copeus* has al-

*Fig. 5.* The mastax muscles of *Trichocerca rattus*. Each muscle cell is numbered; 6 and 3 are the only bicellular muscles present; all the others are unicellular. (a and b) Location of the muscles: they insert on the trophi (stippled) or on the walls of the mastax cavity: the piston muscle (4) on the floor, cells 3, 5, 8 and 12 on the lateral walls. (c) Diagram of the gap junctions present between these muscles. The same symbols are used as in figure 3e. [From Clément & Amsellem, in press].



*Fig. 6.* Neuro-ethological schematic diagram of the feeding behavior in *Trichocerca rattus*, with the succession of three sequences: swimming, crawling, ingestion of food (see text). [From Clément & Amsellem, in press].

ready been analyzed [Clément *et al.*, 1983]. Since then, the study has been extended and an in-depth ultrastructural analysis of the basis of movements in *Trichocerca rattus* has been completed. Here, I shall present only the essential conclusions of a recent summary of this investigation [Clément & Amsellem, 1985].

### a) Coordination of movements in the mastax of T. rattus

All the mastax movements in *T. rattus* seem to be linked (Fig. 5). Clément *et al.* [1983] distinguished 2 separate behavioral sequences: 'attempt at piercing the algal filament' and 'sucking by mastax movements'. With the aid of ultra-thin serial sections, it is possible to count the muscle cells in the mastax (11 paired and 2 unpaired), to pinpoint their insertions, and to detect definite and probable gap junctions between these cells (Fig. 5).

From these observations, J. Amsellem and I have concluded that all the muscle cells seem to be coupled. Therefore, the mastax is only capable of a few specific types of coordinated movements, which translate into piercing or non-piercing of the alga. If piercing is successful it is immediately followed by aspiration of the cell's contents. Usually, several sucking movements are required before the migration of chloroplasts into the pharynx can be seen.

### b) Succession of 3 behavioral sequences: swimming, crawling, and feeding (Fig. 6)

Retraction commences whenever the rotatory apparatus of *T. rattus* comes into contact with an algal filament. If the palpar organ also comes into contact with the algal filament, the animal begins to crawl along it. The palpar organ is a highly specialized tactile receptor [Clément, 1977a, b; Clément *et al.*, 1983]. Crawling involves at least a partial retraction of the cingulum. In *N. copeus* the retraction is made evident by invagination of the auricles which bear the cingulum. In *T. rattus*, there is another muscle (an extension of the central retractor or perhaps a small independent muscle?)

inserted onto cells of the cingulum beside the cingulum muscle which is inserted on the infraciliature (see above and Fig. 2).

Crawling is always accompanied by an attempt at piercing the filament, after a period of time which varies according to several parameters (e.g., age of the animal and its nutritional state). This implies that there is a passage of information from the brain to the mastax ganglion. A nerve which may work in this regard has been described at the level of the light microscope by Remane (1929–32). The mastax then pierces the filament and sucks out its contents. The sucking movements are repeated only if the animal was successful in the first place and the mastax receptors [described by Clément, 1977a; Clément *et al.*, 1983] were stimulated by algal material. If the mastax receptor is not stimulated (because the wall was not pierced, or the algal cell was empty, or the contents of the cell were not appropriate) the sucking movements cease and the animal resumes crawling or, in case of loss of contact with the filament, swimming.

## 6. Other rotifer movements

The purpose of this section is to discuss briefly the motor sequences of other behaviors which have not been analyzed to the same extent as the ones described already.

### a) The feeding behavior of Brachionus calyciflorus and other rotifers having a malleate or ramate mastax

Gilbert and Starkweather [1977] have described three mechanisms by which the ingestion of suspended particles is regulated in *B. calyciflorus*. The cirri of the pseudotrochus can assume one of 2 positions: extension, allowing particles to enter, or screen formation, preventing particles (even as small as yeast) from entering. Plate III shows that each cirrus is composed of dozens of closely packed cilia which have a highly developed infraciliature. The latter consists of striated ciliary rootlets which extend deep into the epithelial cell

and serve for the insertion of muscles (Plate IIId).

The assemblage of muscles, infraciliature, and cilia can partly explain the control of the quality of ingested food in the case of the pseudotrochus. Nevertheless, the scheme suggested by Clément and Pourriot [1982] and Clément [1983] remains to be verified through the identification of the sensory receptors involved and of the muscles that insert onto the pseudotrochus.

The two other mechanisms observed by Gilbert and Starkweather still await confirmation and analysis. They involve a rejection of particles from the oral canal 'presumably by a change in ciliary beat' and a rejection by the jaws which push the particles back into the buccal field.

The motor mechanisms involved in feeding, doubtless show similarities in all rotifers having a grinding (malleate or ramate) type of mastax. The presence of a buccal velum which we have observed in *B. calyciflorus*, *B. plicatilis*, *Philodina roseola* and, most recently, *Filinia longiseta* supports this hypothesis.

### b) Substract attachment by means of the foot

To my knowledge, motor sequences leading to the attachment of a rotifer to the substrate have not been described with precision. However, the sensory determination of attachment has been studied by Viaud [1947] in *Brachionus rubens* and by Wallace [1975, 1978, 1980] in sessile rotifers [reviewed in Clément et al., 1983].

Electron microscopic examination of the foot has shown that the ducts of the pedal glands are surrounded by a muscular envelope [Clément, 1977a, b] and that these muscles are innervated [Amsellem & Clément, unpubl. obs.]. In *T. rattus*, the pedal glue is secreted by the toes at the base of the tail. At first, it accumulates in a reservoir whose walls are lined by muscles characterized by a slow, protracted, contraction [Clément, 1977a, b; Clément & Amsellem, unpubl. obs.].

### c) Copulatory behavior

Ethological or neuro-ethological studies are lack-ing for this behavior. There seem to be some sensory receptors at the tip of the penis [Clément et al., 1983]. The copulatory behavior of male *B. calyciflorus* has been described by Gilbert [1963] in the presence and absence of the female and filmed by Clément and Pourriot [1982] along with those of *N. copeus* and *A. brightwelli*. Males and females come into contact at random as they move about. In the case of *B. calyciflorus* the chances of contact are increased through a chemoklinotaxis [Gilbert, 1963]. However, it is not known what motor sequences intervene between the initial contact and the attachment of the penis to the integument of the female.

### d) Oviposition

Oviposition has been described and filmed [Pourriot & Clément, 1982]; we don't know which muscles are involved in this activity. A muscular sphincter around the cloacal opening has not been detected using electron microscopic techniques [Clément et al., 1983]. In *T. rattus* there is a longitudinal muscle whose contraction seems to aid opening the cloacal aperture. It is not known which glands secretes the filament that anchors eggs to the foot of the female in those species that carry their eggs around.

In *N. copeus*, egg laying is followed by a distinctive behavior sequence [Clément, 1977a; Pourriot & Clément, 1982; Clément et al., 1983]. A neuroethological analysis of this behavior is lacking. Such an analysis would be all the more interesting since, of all the rotifer activities described to date, this is one of the longest (aroudn 20 minutes) and most elaborate.

### 7. Conclusions

Analysis of rotifer behavior is still in its infancy. Therefore, it is very risky to ask such questions as: Why do these behaviors exist? and Why do these movements take place? Of course, the very fact that these behaviors exist indicates that they have some adaptive significance as they have withstood natural selection. As I have mentioned in the introduction, these behaviors are sufficiently well adapted

to the environment so that the species or clones inhabiting that environment are able to survive.

Rather than succumbing to the illusion of providing an answer by evoking the term 'optimalization', a modern substitute for finalism, I wish to turn the question around and ask 'why not?' as a challenge to rotiferologists and biologists in general. Investigators should make use of the extraordinary simplicity of rotifer structure and behavior, and examine the problems of evolution by means of a comparative study of rotifer behavior. These animals possess highly advantageous characteristics, particularly isogeny resulting from diploid parthenogenesis [King, 1977]. They merit consideration by a joint team of ecologists, population geneticists, ethologists and neurobiologists. Such a collaborative venture ought to result in a description of behavior in terms of the life history of the individuals, of the populations and of the species.

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