TETRAHYMENA DIMORPHA SP NOV. (HYMENOSTOMATIDA: TETRAHYMENIDAE), A NEW CILIATE PARASITE OF SIMULIIDAE (DIPTERA) WITH POTENTIAL AS A MODEL FOR THE STUDY OF CILIATE MORPHOGENESIS

By B. S. BATSON

Department of Zoology, University College of Wales, Penglais, Aberystwyth SY23 3DA, U.K.

[Plates 1–7]

CONTENTS

A new species of hymenostome ciliate, *Tetrahymena dimorpha* sp.nov., is described. This ciliate occurs as a parasite in the haemocoel of larval, pupal and adult Simuliidae (Diptera). In larval hosts the total number of parasites never exceeds about 240 and the infection is benign. Within larval hosts the ciliates are large and broadly oval and possess an unusually wide range of somatic kineties, from 30 to 66; moreover a variable proportion of these kineties are characteristically disorganized, being incomplete, meandering or branched. Metamorphosis of the host to the adult fly is accompanied by a dramatic increase in the number of ciliates, which reach pathogenic intensity. Adult hosts may contain up to 19000 ciliates and the flies soon die from this

heavy burden. Associated with ciliate population growth during host metamorphosis is a startling morphological transformation of the ciliates themselves. In adult hosts the ciliates are smaller and pyriform in shape and the cortex is greatly modified; the total number of somatic kineties is considerably reduced and has a limited range of 19–22. Most significantly, the kineties are ordered with typical tetrahymenine precision. By application of appropriate culture conditions to ciliates isolated from any host stage, either of the distinctive morphological forms of *T. dimorpha* may be selectively induced *in vitro*. In bacterized infusions, ciliates are produced that have the general form and cortical characteristics of those found naturally in adult hosts. Sterile culture in serum-supplemented Mitsuhashi and Maramorosch insect tissue culture medium produces a population showing features characteristic of ciliates from larval hosts. Sterile culture in proteose–peptone–yeast-extract medium results in populations exhibiting concurrent dimorphism, even after cloning. The extreme nature and multiple facets of the dimorphism together with the ease of its manipulation *in vitro* afford opportunities for the experimental investigation of many problems, particularly those related to cell surface patterning in ciliates, and these possibilities are discussed in relation to current concepts of ciliate morphogenesis.

INTRODUCTION

Ciliated protozoans have been used effectively in the study of fundamental problems in biology (Beale 1977). In particular hymenostome ciliates of the *Tetrahymena pyriformis* complex are widely employed in biochemical, developmental and genetic contexts (Elliott 1973; Nanney 1980). All *Tetrahymena* cell lines in common use have been derived from free-living ciliates, but a number of species in this genus exhibit parasitic tendencies towards metazoan organisms, primarily invertebrates and particularly dipterous insects (Corliss 1973). Preliminary investigations upon tetrahymenids parasitic in simuliid and megalopteran hosts have convinced the author of the great potential value of these ciliates, particularly for studies upon development. The aim of the present paper is therefore to focus attention upon one such ciliate, to establish pertinent basic information upon which future work may be founded, and to discuss the value of this organism as a new tool for the experimental ciliatologist. The selected organism is a new species of hymenostome ciliate parasitic in Simuliidae (Diptera), for which the name *Tetrahymena dimorpha* sp.nov. is proposed. This choice of ciliate was made for a number of reasons. First, *T. dimorpha* manifests in its natural life cycle a remarkable dimorphism. Secondly, this ciliate may be readily cultivated *in vitro*, where manipulation of culture conditions can induce selective or concurrent expression of the two morphological forms. Thirdly, one conspicuous aspect of the dimorphism *in vivo* and *in vitro* is a most un-ciliate-like lack of precision in the replication of cortical pattern. Fourthly, the ciliate possesses a micronucleus, conjugation has been observed and the possibility of genetic examination and manipulation exists. Finally, as a parasite *T. dimorpha* exhibits a subtlety of association with its simuliid host that is not seen in other insect-parasitic ciliates.

MATERIALS AND METHODS

Collection and maintenance of hosts

Larval and pupal simuliids were collected from the River Wey near Tilford in Surrey, where they occurred in abundance attached to aquatic plants, primarily *Sparganium* sp. and *Ranunculus* sp. Great care was taken to avoid stressing larvae and pupae during transfer to the laboratory; they were transported at low density in plastic containers of fresh river water, and maintained at 5–10 °C during transit. In the laboratory, larvae were maintained in aerated river water at

5, 10 or 15 °C. Transmitted-light microscopy at magnifications of × 100 or more was necessary to determine the presence or absence of ciliates in the larval haemocoel. Some infected larvae were transferred to a laboratory simuliid rearing system (Grunewald 1973) for completion of the life cycle.

Samples of mature larvae and pupae were preserved for identification according to the key of Davies (1968). Immature larvae cannot be identified with certainty.

Establishment and maintenance of axenic and non-axenic cultures

Infusions

Infected larvae, pupae and adult simuliids were briefly surface-sterilized in 70 % ethanol and then vigorously washed in several changes of distilled water. Hosts were then teased open in 3–5 ml of Prescott and James solution (Prescott & James 1955) in a small Petri dish to release the ciliates. After removal of the host cadaver a few grains of rice or flakes of wheatgerm were added. Infusions were maintained at 15–20 °C and subcultured at 6–8 week intervals.

For convenient microscopical observation of ciliates following release from the host, 'slide infusions' were used. The procedure followed that outlined above except that hosts were teased open on a microscope slide and only a few drops of Prescott and James solution were necessary. Before addition of a cover slip most of the host cadaver was removed but the small fragments remaining, together with the diluted body fluids, provided an excellent infusion medium. Such slide infusions were kept in humid chambers to prevent evaporation.

Axenic culture

Infected larvae, pupae and adult simuliids were surface-sterilized in 70 % ethanol and then vigorously washed in several changes of sterile distilled water. Following transfer to sterile culture medium the host's abdominal wall was carefully breached with the aid of very fine tungsten needles. Gentle pressure on the host's abdomen produced a stream of haemolymph containing ciliates. To ensure sterility, ciliates were then transferred with micropipettes through a series of three culture medium washes before final transfer to tissue culture flasks. The whole operation was carried out under a dissecting microscope in a horizontal laminar-flow sterile cabinet, and sterile technique was observed throughout.

Axenic cultures were maintained at 10, 15 and 20 °C with routine subculture at 5–20 day intervals. The following media were used:

(i) Proteose–peptone–yeast-extract; proteose–peptone (Difco) 10 g l^{-1}, yeast-extract (Difco) 2.5 g l^{-1};

(ii) Mitsuhashi and Maramorosch insect tissue culture medium (Gibco);

(iii) Mitsuhashi and Maramorosch insect tissue culture medium supplemented with 20 % foetal calf serum (Gibco);

(iv) Mitsuhashi and Maramorosch insect tissue culture medium supplemented with 20 % newborn calf serum (Gibco);

(v) Grace's insect tissue culture medium (Gibco);

(vi) Grace's insect tissue culture medium supplemented with 20 % foetal calf serum;

(vii) Grace's insect tissue culture medium supplemented with 20 % newborn calf serum.

No antibiotics were used during initial isolation or during subsequent subcultivation. All initial isolations were made into proteose–peptone–yeast-extract medium with transfer to other media, through graded mixtures, as required.

For cloning purposes, single cells were transferred with micropipettes via a series of culture medium droplets (in order that positive verification of single cell transfer could be obtained) to fresh medium in a new culture vessel.

Light microscopy

Living and preserved ciliates were observed and photographed with a Leitz Dialux 20 photomicroscope under a variety of optical conditions, including bright field, dark field, phase contrast and Nomarski interference contrast. Photographs of living specimens were obtained with the aid of an automatic microflash (Batson & Lloyd 1981). Measurements were made with the aid of a Leitz ocular graticule.

Ciliates from infected simuliids, from infusions and from axenic culture were prepared for silver impregnation by the Chatton–Lwoff technique (Corliss 1953). Nuclear characteristics were revealed by means of the Feulgen nucleal reaction (Pearse 1968). Air-dried smears of ciliates were stained with oil-red-O or Sudan-black-B (Pearse 1968), smears extracted with chloroform:methanol (2:1) being used as controls.

Scanning electron microscopy

Ciliates from naturally infected hosts and from axenic culture were layered upon 0.45 or 3.0 μm Millipore filters, washed thoroughly with Clark's insect saline (Hale 1976) and then fixed with 3% glutaraldehyde in 0.1 M Dulbecco's phosphate buffer at pH 7.1 for 1 h at room temperature. After washing in buffer, secondary fixation was in osmium tetroxide solution (1 g/100 ml) in the same buffer as before for a further 1 h at room temperature. Following dehydration in a graded ethanol series, specimens were transferred from absolute ethanol through an ethanol:fluorisol series to absolute fluorisol, then dried in a Polaron E3000 critical-point-drying apparatus. The Millipore filters were mounted on metal stubs, coated with gold in a Polaron E5000 coating unit and examined in a Cambridge 150 scanning electron microscope.

RESULTS

Host data

At those sampling stations selected on the River Wey near Tilford in Surrey the simuliid population was found to comprise only two species, *Simulium equinum* and *S. ornatum*.

Periodic sampling over a $2\frac{1}{2}$ year period showed that, although the incidence of infection varied considerably, nevertheless the presence of parasitic ciliates was a consistent feature of the larval simuliid population (table 1). Overall incidence of infection in larval simuliids is somewhat misleading as it does not take account of the host specificity of *Tetrahymena dimorpha*. During the study period a total of approximately 300 infected hosts were sufficiently mature to be positively identified, that is, they were mature larvae, pupae or adults. All hosts were *S. equinum* with the exception of a single infected *S. ornatum* larva. This apparent tendency towards host specificity is reflected in the elevated incidence of infection when *S. equinum* larvae are considered separately (table 1). In this way the maximum incidence of infection was recorded to be 52% compared with 34.85% for the population as a whole. Caution is clearly necessary in the interpretation of such field data; slight retardation of host development for example might result in the persistence of infected larvae beyond the time when most uninfected larvae

would normally have pupated, thus causing an apparent elevation in the incidence of infection in those larvae remaining.

In an attempt to determine whether *T. dimorpha* occurred naturally as a parasite of other aquatic invertebrates a total of approximately 600 larval insects representing Chironomidae, Plecoptera, Trichoptera and Ephemeroptera were carefully examined for the presence of parasitic ciliates. Collection of these insects was spread throughout the study period and they were recovered from the same sampling stations, and often from the same piece of water weed, as infected Simuliidae. All examinations were negative.

TABLE 1. INCIDENCE OF *TETRAHYMENA DIMORPHA* SP.NOV. INFECTIONS IN LARVAL AND PUPAL SIMULIIDS FROM THE RIVER WEY

	all larvae		*Simulium equinum* larvae		*Simulium equinum* pupae	
	percentage infected	n	percentage infected	n	percentage infected	n
Sept. 1979	27	100	0	0	0	11
Oct. 1979	15.9	151	0	0	0	0
Jan. 1980	26.3	167	46	13	20	5
Mar. 1980	34.9	175	52	115	26	27
Aug. 1980	4.3	231	6.9	29	2	53
May 1981	4.2	96	3	66	1	102
Feb. 1982	5.8	139	13.3	45	0	58

Simuliid larvae infected with *T. dimorpha* were recorded from a 10 km stretch of the River Wey, with the village of Tilford marking the midpoint. Determination of a more widespread distribution within and beyond the Wey system was not attempted, but it should be noted that extensive sampling of simuliid larvae throughout the mid-Wales river systems over a number of years by the author has failed to reveal even a single incidence of ciliate parasitism.

Other pathogens infecting simuliid larvae in the River Wey included mermithid nematodes, microsporidian Protozoa, and the chytrid fungus *Coelomycidium simulii*. Joint infections of *T. dimorpha* with each of these organisms were occasionally recorded.

Attempts to infect laboratory-reared simuliid larvae with *T. dimorpha* have so far been unsuccessful and naturally infected hosts provide no evidence concerning the precise mode of infection, but observations on field-collected larvae suggest that they become infected during their early instars. The possibility that larvae might be infected transovarially seemed unlikely in view of the pathogenicity of *T. dimorpha* in adult simuliids (see 'Parasites in pupal and adult hosts', below); nevertheless many hundreds of freshly hatched larvae from field-collected egg masses were screened during the study period, and not a single infection was detected. It seems probable therefore that re-infection of successive generations of simuliid larvae is accomplished by free-living ciliates; *T. dimorpha* from all stages of the host are able successfully to assume a free-living existence (see 'Free-living ciliates', below).

Infected larvae are able to pupate normally and subsequently to produce adult flies, although metamorphosis of the host is accompanied by dramatic quantitative and qualitative changes in the population of parasitic ciliates, as described below.

Parasites in larval hosts

In larval, pupal and adult Simuliidae *Tetrahymena dimorpha* occurred free in the haemocoel. The essentially transparent ventral body wall of larvae allowed observation of living parasites

within all parts of the body cavity (figure 1†) including the head capsule. Live ciliates in larval hosts were broadly oval (figure 2), but were capable of considerable deformation when actively moving between the internal organs of the host. The cytoplasm was densely packed with inclusions which rendered the ciliates conspicuously opaque in transmitted light (figures 1, 2). A translucent central region marked the position of the macronucleus (figure 2). A large proportion of the cytoplasmic granules stained strongly with oil-red-O and Sudan-black-B, indicating the presence of significant quantities of lipid. In larval hosts dividing ciliates were seen only rarely. Evidence of conjugation was never observed.

Late instar larvae bore a mean intensity of infection of 147 ± 52 ciliates (70–240, $n = 50$), but no larva was ever observed to contain more than approximately 240 ciliates, even mature larvae preparing to pupate. In general, younger larvae were host to fewer ciliates than more mature larvae. Small larvae containing just one or two ciliates were considered to be recently infected, and the ciliates in these cases were noticeably translucent, lacking the cytoplasmic inclusions described earlier. The cytoplasmic inclusions quickly became apparent, and by the time that eight or ten ciliates were present the latter had become characteristically opaque.

Development of infected larvae was apparently unaffected by the low parasite burden. No behavioural or morphological abnormality was detected that might have allowed discrimination between infected and uninfected larvae with the naked eye. The ability of infected larvae to pupate normally was unimpaired.

Cortical features

Silver-stained preparations of ciliates from larvae reveal a cortical structure that, while typically tetrahymenine in some respects, exhibits a number of remarkable features. The main morphometric characteristics of silver-stained specimens are summarized in table 2.

The oral apparatus is organized in typical tetrahymenine fashion, accommodating three ciliary membranelles on its left side (figure 3) while its right side is delineated by an undulating membrane (figures 8, 9).

† All figures in this paper appear on plates.

DESCRIPTION OF PLATE 1

Light micrographs of live and silver-stained *Tetrahymena dimorpha* sp.nov. (Hyde form) from larval *Simulium equinum*.

FIGURE 1. Posterior region of living *S. equinum* larva, showing *T. dimorpha* (some indicated by arrows) in the haemocoel. Scale bar, 0.5 mm.

FIGURE 2. Detail of individual *T. dimorpha* moving between two muscle strands (m.s.) within the living host. The central translucent area indicates the position of the macronucleus. Microflash. Scale bar, 50 μm.

FIGURES 3–9. Silver-stained preparations of Hyde cells from larval hosts.

FIGURE 3. Optical section through oral apparatus showing the three membranelles (m.1, m.2, m.3). Scale bar, 10 μm.

FIGURE 4. Hyde cell illustrating variety of cortical disorganization. Kineties are branched (b.k.), fragmented (f.k.), meandering (m.k.) or truncated (t.k.), and the kinetosomes are sometimes irregularly spaced (k.i.) or isolated (arrow). Scale bar, indicating 20 μm, applies also to figures 5–9.

FIGURE 5. Cell with relatively ordered cortex showing few deviations.

FIGURES 6 AND 7. Cells in which cortical disorganization leads to difficulties in distinguishing individual kineties. Notice the variation in interkinetal spacing between these two cells.

FIGURES 8 AND 9. Slightly different optical sections of a single anteroventral orientated specimen revealing the undulating membrane (u.m.) of the oral apparatus (figure 8). the pre-oral suture (p.s.) and the anterior 'bald' patch devoid of kinetosomes (figure 9).

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u.m.
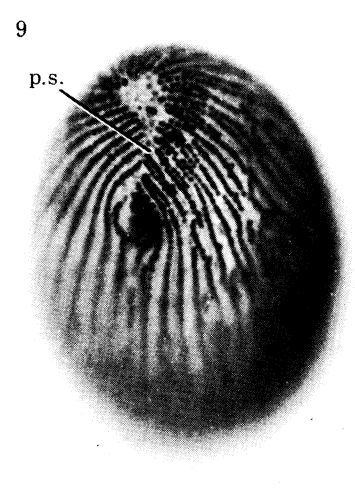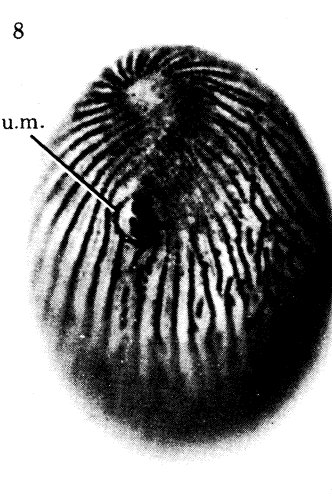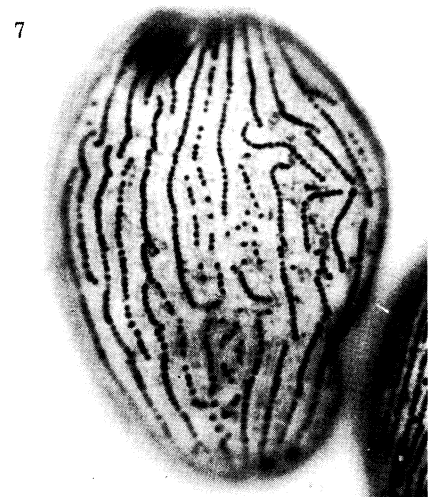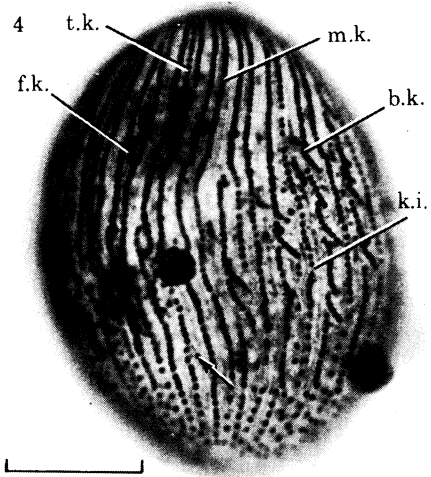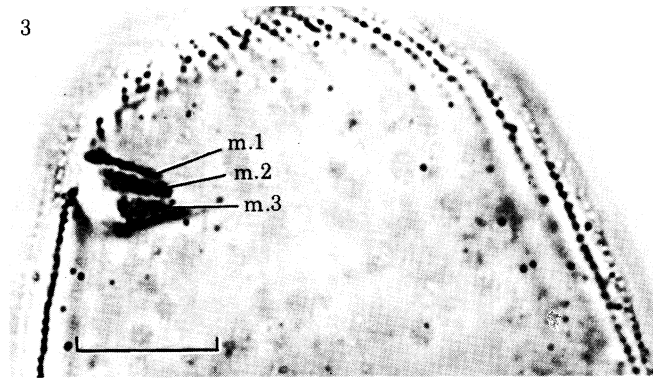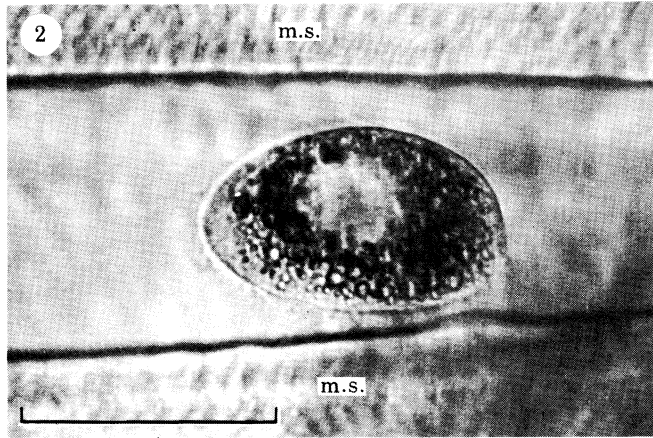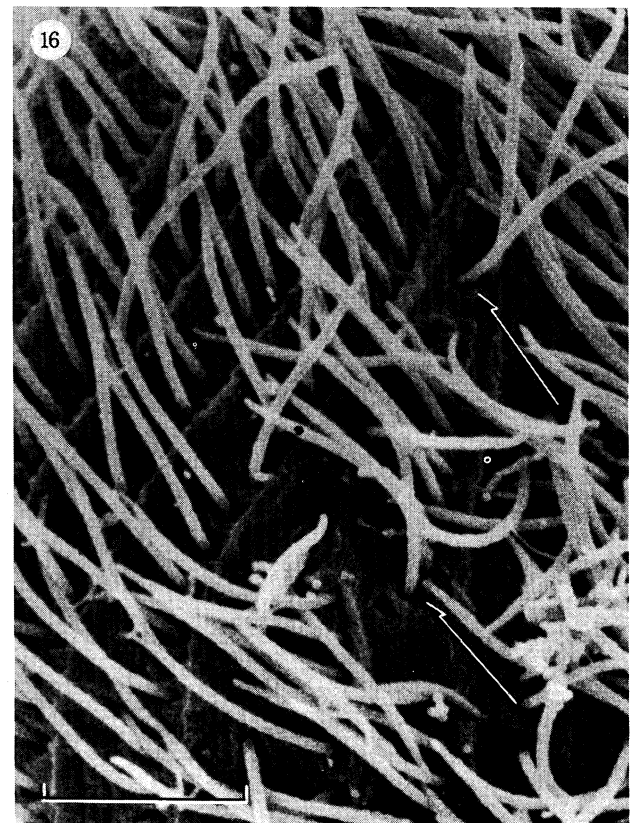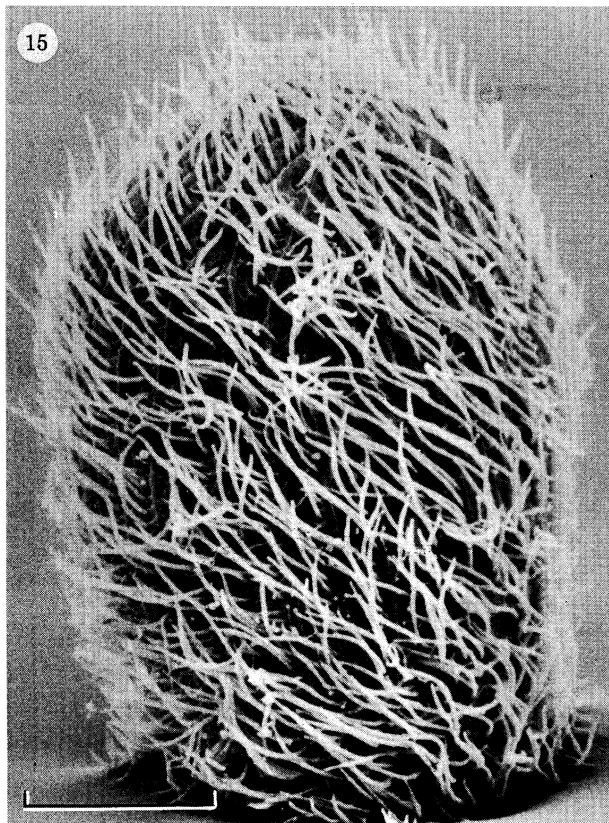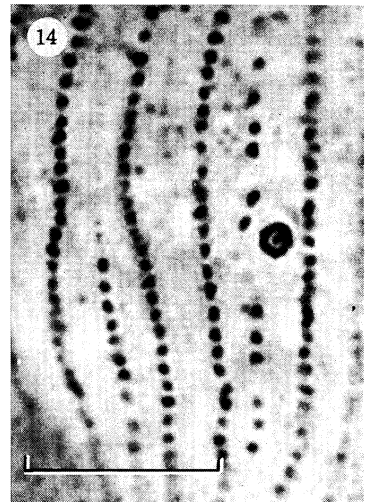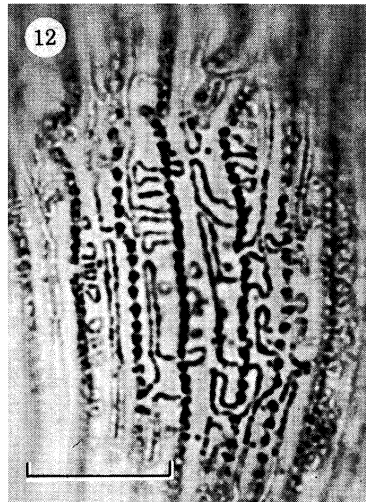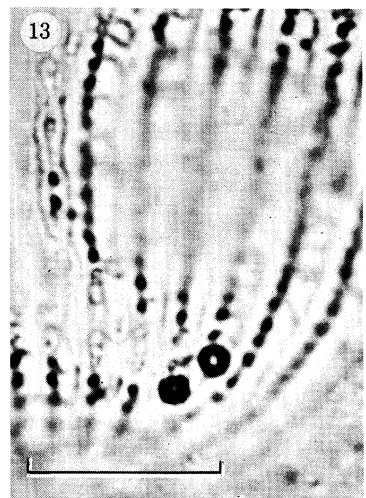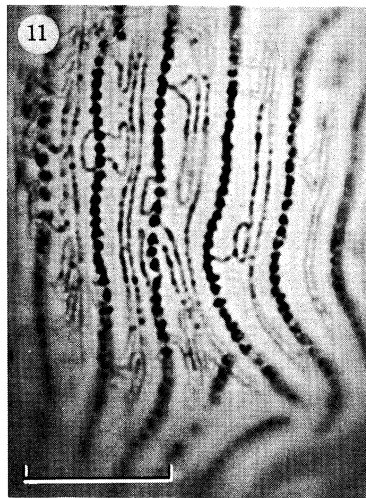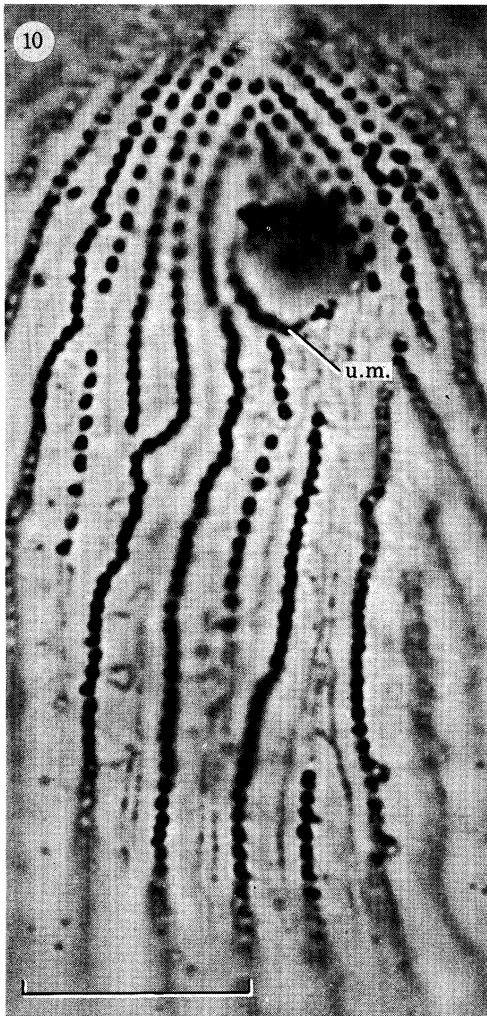
DESCRIPTION OF PLATE 2

FIGURES 10–14. Light micrographs showing cortical details of silver-stained Hyde cells from larval *S. equinum*.

FIGURE 10. Oral region illustrating difficulty in quantifying the number of post-oral kineties. Notice the high density of kinetosomes along most kineties, even towards their extremities. The oral apparatus membranelles are not in the focal plane. Abbreviation u.m. stands for undulating membrane. Scale bar, 10 μm.

FIGURES 11 AND 12. Detail of cortex showing looped and twisted configuration of the secondary meridians. Notice also the densely packed kinetosomes of the primary meridians. Scale bar, 10 μm.

FIGURE 13. Two contractile vacuole pores in normal positions at the posterior ends of kineties 7 and 8. Scale bar, 10 μm.

FIGURE 14. Abnormally positioned contractile vacuole pore in the equatorial region of a cell not undergoing stomatogenesis. Scale bar, 10 μm.

FIGURES 15 AND 16. Scanning electron micrographs of *T. dimorpha* from larval *S. equinum*.

FIGURE 15. Single Hyde cell showing general body form and dense somatic ciliature. Scale bar, 10 μm.

FIGURE 16. Detail of cortex showing region of kinetal disarray (arrows). Notice also the close proximity to each other of cilia in the adjacent, apparently normal, kineties. Scale bar, 4 μm.
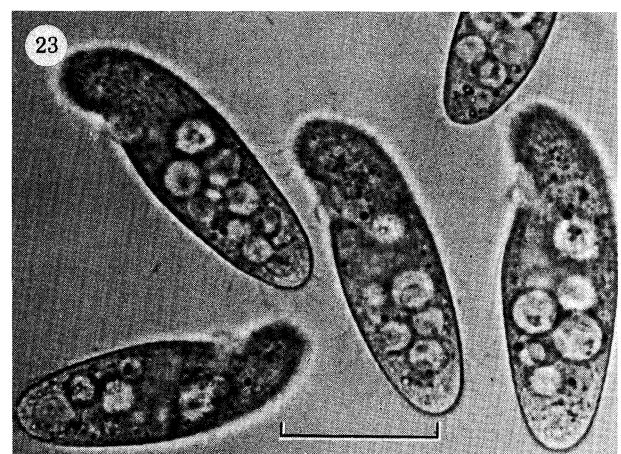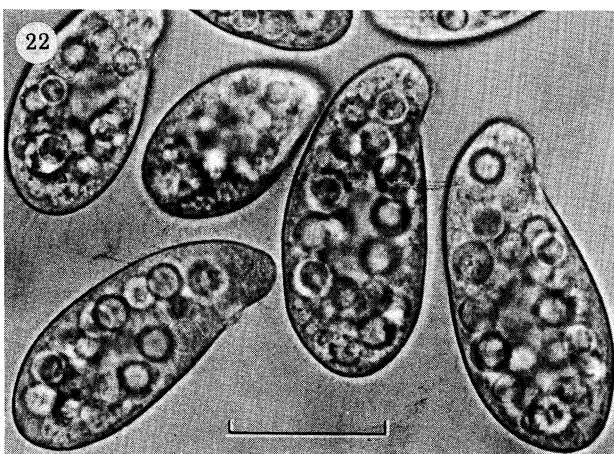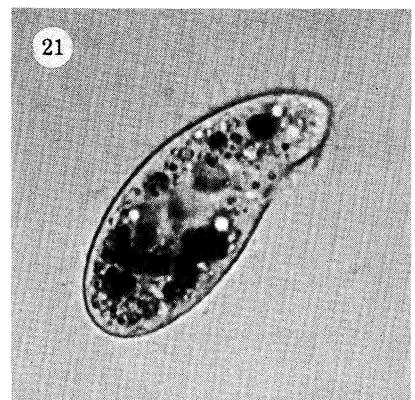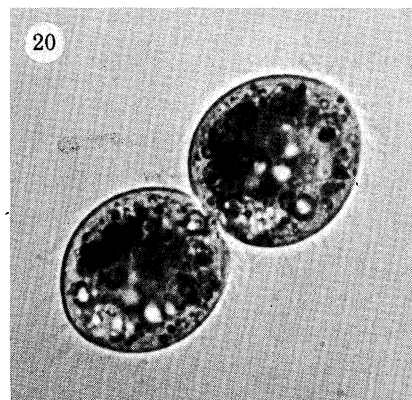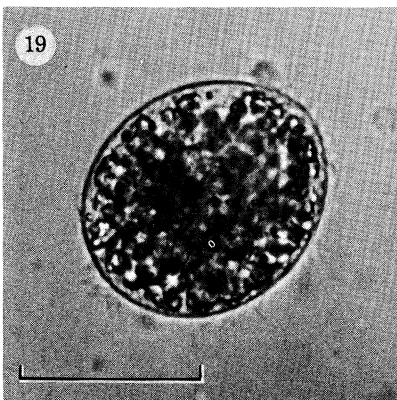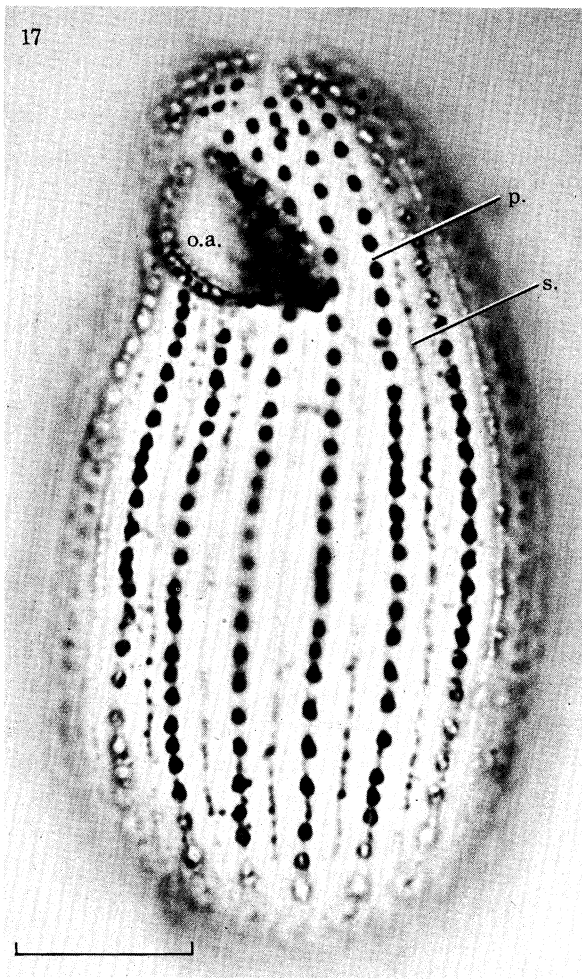
DESCRIPTION OF PLATE 3

FIGURE 17. Light micrograph of silver-stained *T. dimorpha* sp.nov. from adult *S. equinum* showing the ordered primary (p.) and secondary (s.) meridians of the Jekyll form. Notice the more elongate shape of the cell compared to the Hyde form, and the presence of two short pre-oral kinetal segments anterior to the oral apparatus (o.a.). Scale bar, 10 μm.

FIGURE 18. Scanning electron micrograph of Jekyll form of *T. dimorpha* from adult *S. equinum*. Notice the single incomplete kinety (arrow). Scale bar, 10 μm.

FIGURES 19–21. Decrease in cytoplasmic granularity during transition from parasitic to free-living ciliate. Living specimens. Microflash. Scale bar, indicating 30 μm, applies to all three figures.

FIGURE 19. Ciliate 3 h after release from adult host into Prescott and James solution at 15 °C. Notice the swollen appearance, which is due to osmotic stress.

FIGURE 20. Division of ciliate 8 h after release from the host.

FIGURE 21. After 20 h post-release, cytoplasmic granularity decreases and the typical free-living body form is established.

FIGURE 22. *T. dimorpha* from 3 day old infusion derived from larval parasites. The cytoplasm is packed with food vacuoles. Microflash. Scale bar, 30 μm.

FIGURE 23. Showing more slender body form of ciliates from an older (12 day) infusion. Microflash. Scale bar, 30 μm.

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Batson, plate 4

c.v.p.
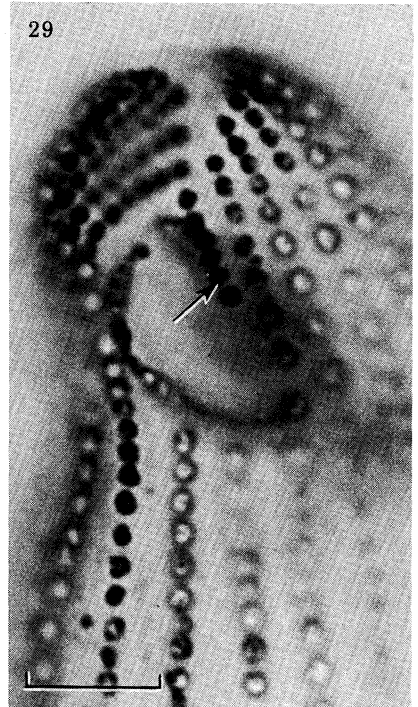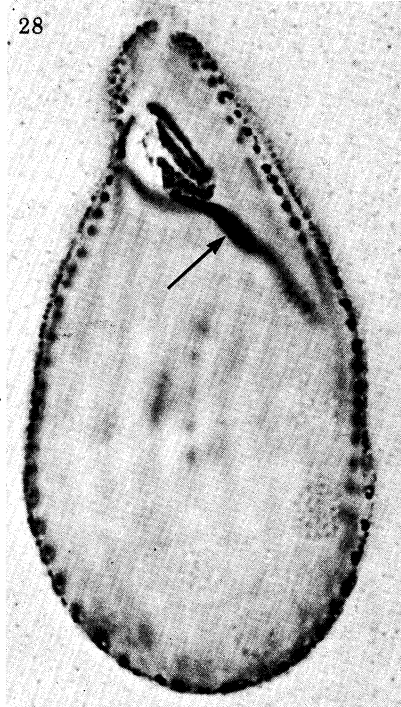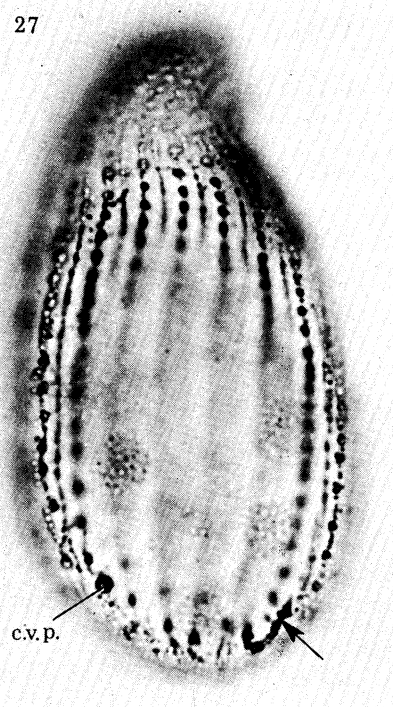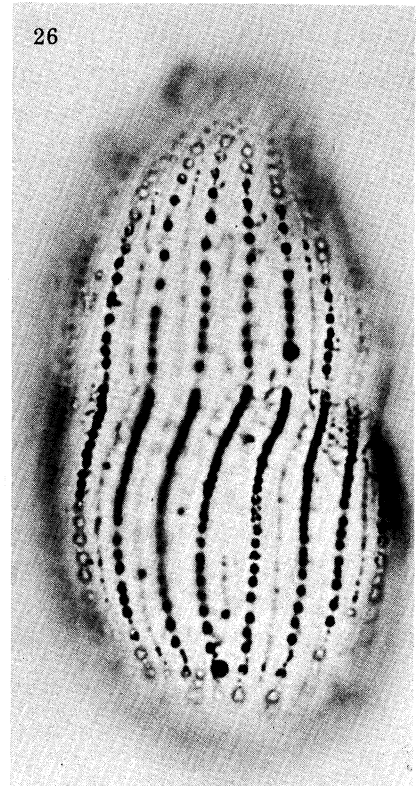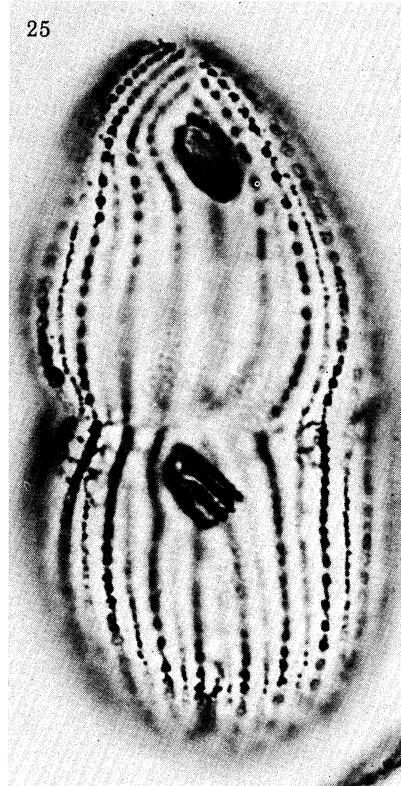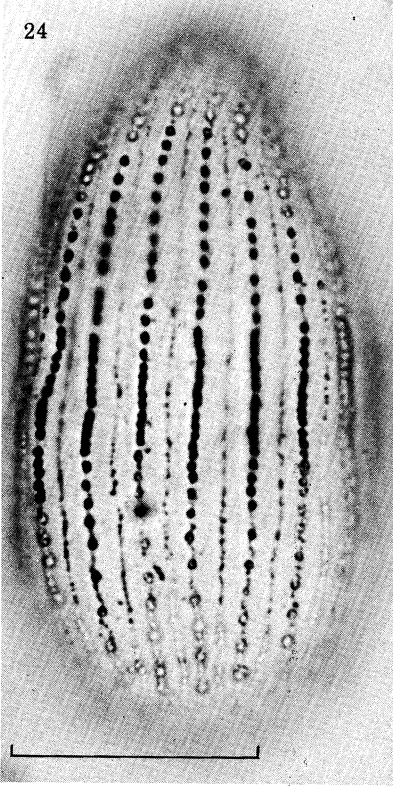
Light micrographs of Jekyll cells: silver-stained preparations of free-living ciliates from bacterized rice infusions. Notice particularly the highly ordered arrangement of cortical organelles in all specimens.

FIGURE 24. Dorsal surface of ciliate nearing cell division. Scale bar, indicating 20 μm, applies also to figures 25–28.

FIGURE 25. Ventral surface of ciliate nearing cell division, showing formation of new oral apparatus and division furrow.

FIGURE 26. Dividing cell which has produced a new contractile vacuole pore on a different kinety in the proter to that bearing the original in the opisthe.

FIGURE 27. Lateral view of non-dividing cell, showing relative positions of contractile vacuole pore (c.v.p.) and cytoproct (arrow).

FIGURE 28. Optical section of oral apparatus to show the three membranelles corresponding to those in figure 3. The 'deep-fibre' system (arrow) is particularly conspicuous.

FIGURE 29. Detail of oral apparatus region, illustrating a single pre-oral kinetal segment composed of six kinetosomes (arrow). Scale bar, 5 μm.
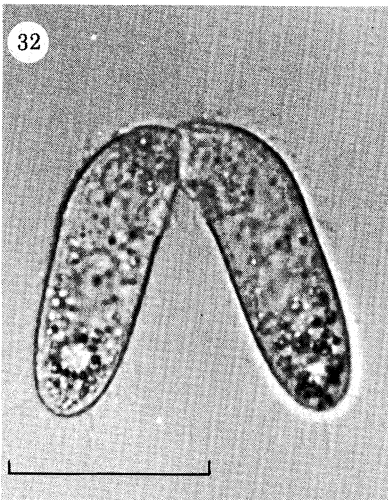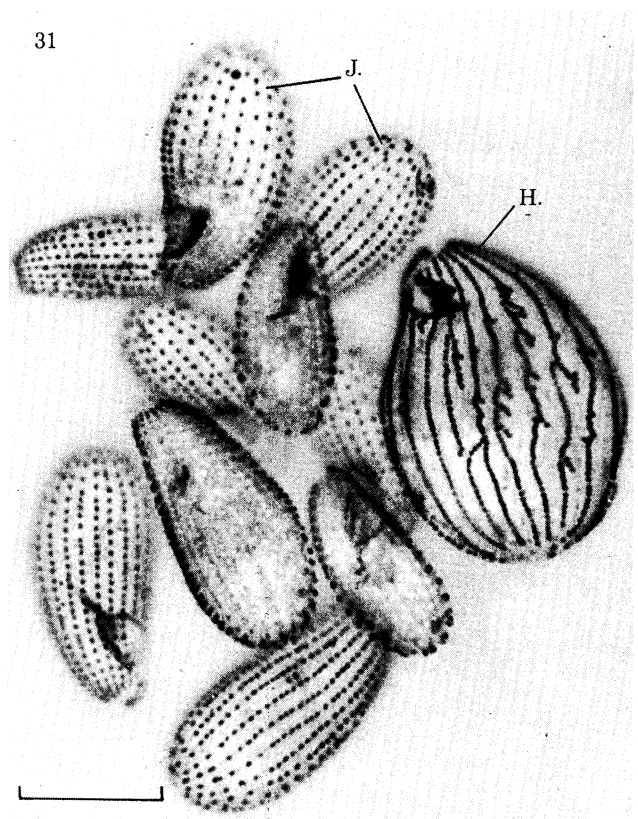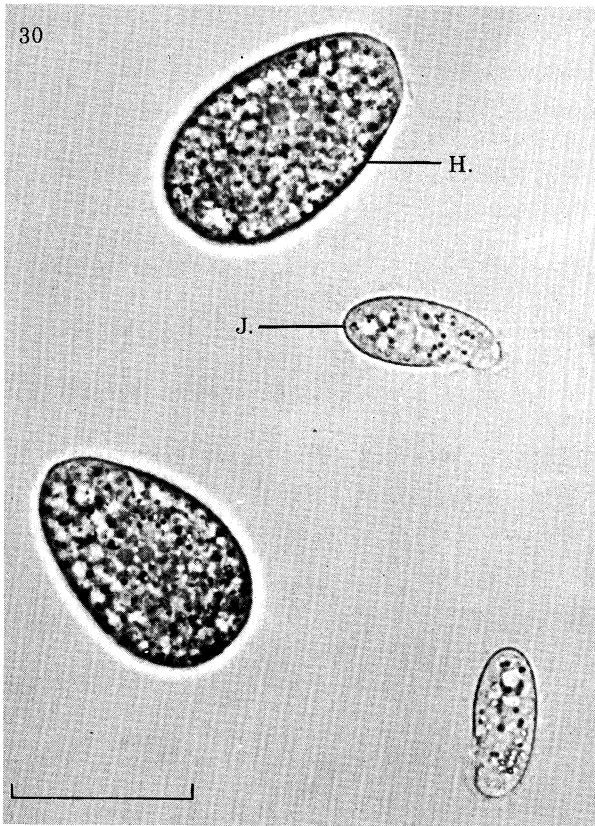
DESCRIPTION OF PLATE 5

Light micrographs of living and silver-stained *T. dimorpha* sp.nov. exhibiting concurrent dimorphism in proteose–peptone–yeast-extract medium.

FIGURE 30. Live ciliates of both Jekyll (J.) and Hyde (H.) types; notice the distinctive size, shape and opacity of each type. This culture was clonal, having been initiated from a single Hyde cell. Microflash. Scale bar, 40 µm.

FIGURE 31. Silver-stained preparation showing a single Hyde cell (H.) and a cluster of Jekyll cells (J.). Scale bar, 20 µm.

FIGURE 32. Live conjugating pair of Jekyll cells. Microflash. Scale bar, 30 µm.

FIGURES 33–36. Silver-stained preparations of Hyde cells from proteose–peptone–yeast-extract culture, illustrating a wide range of cortical disorganization from slight (figure 36) through intermediate conditions (figures 33, 34) to severe (figure 35). Notice the position of contractile vacuole pores (arrows) at the posterior end of a single incomplete kinety in figure 36. Scale bar, indicating 20 µm, applies to all figures.

FIGURE 37. Possible intermediate stage (cell on the right) in the transition from Jekyll to Hyde or Hyde to Jekyll. The cell is much smaller than the adjacent typical Hyde cell and has less densely packed kinetosomes, yet possesses some kinetal abnormalities. Scale bar, 20 µm.

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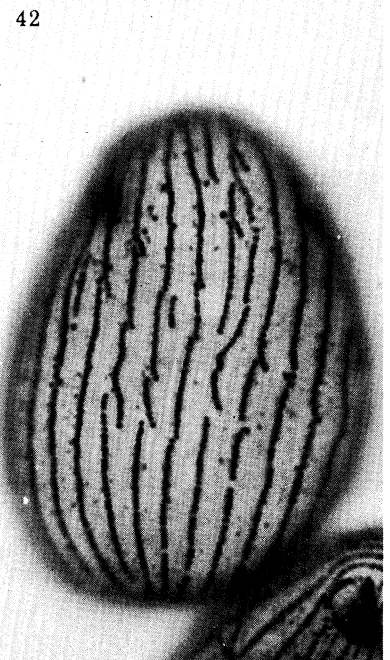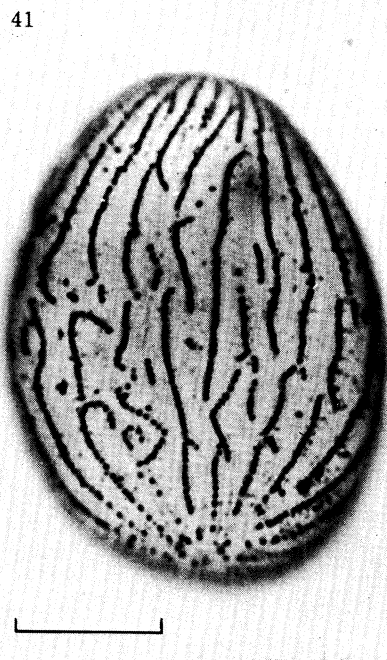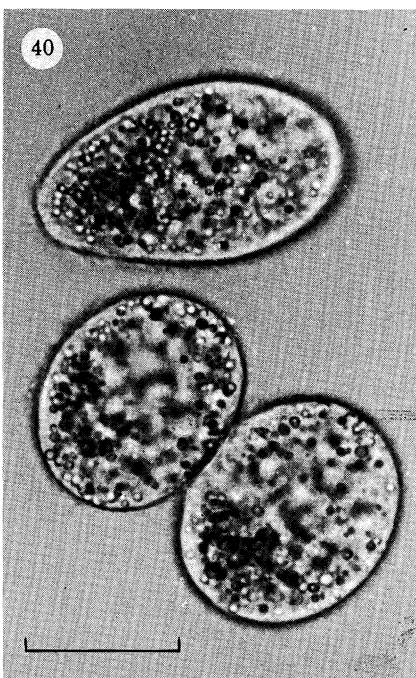
Batson, plate 6

FIGURES 38 AND 39. Scanning electron micrographs of Hyde (figure 38) and Jekyll (figure 39) cells cultured in sterile proteose–peptone–yeast-extract medium, illustrating the contrasting nature of their respective shape, size and density of somatic cilia. Scale bar, 5 μm.

FIGURES 40–42. Light micrographs of live and silver-stained *T. dimorpha* cultured in sterile Mitsuhashi and Maramorosch insect tissue culture medium, supplemented with 20 % foetal calf serum.

FIGURE 40. Living ciliates showing typically Hyde characteristics of cell size, shape and granularity. Microflash. Scale bar, 30 μm.

FIGURES 41 AND 42. Silver-stained preparations illustrating the range of kinetal disorder from slight (figure 42) to severe (figure 41). Scale bar, 20 μm.

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Batson, plate 7
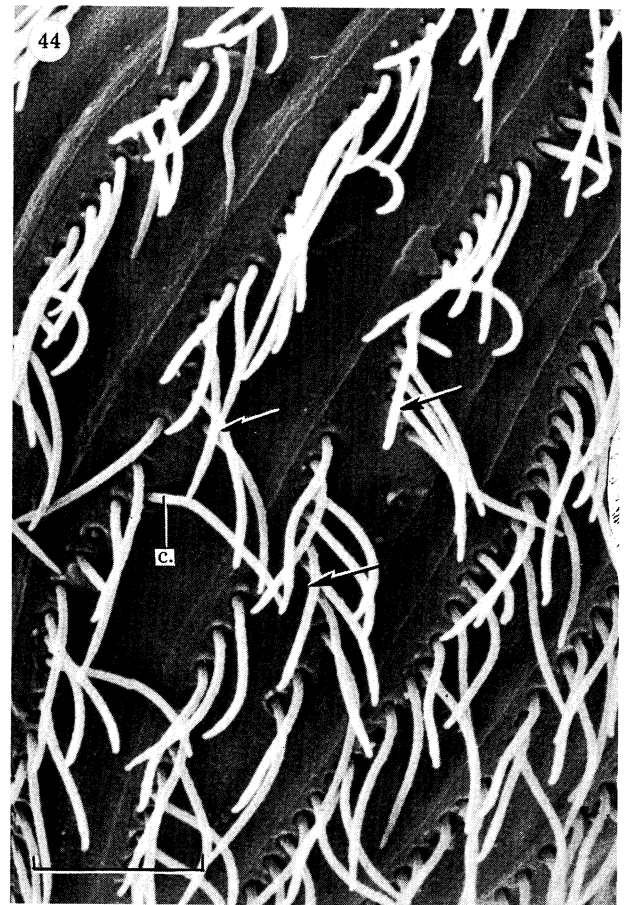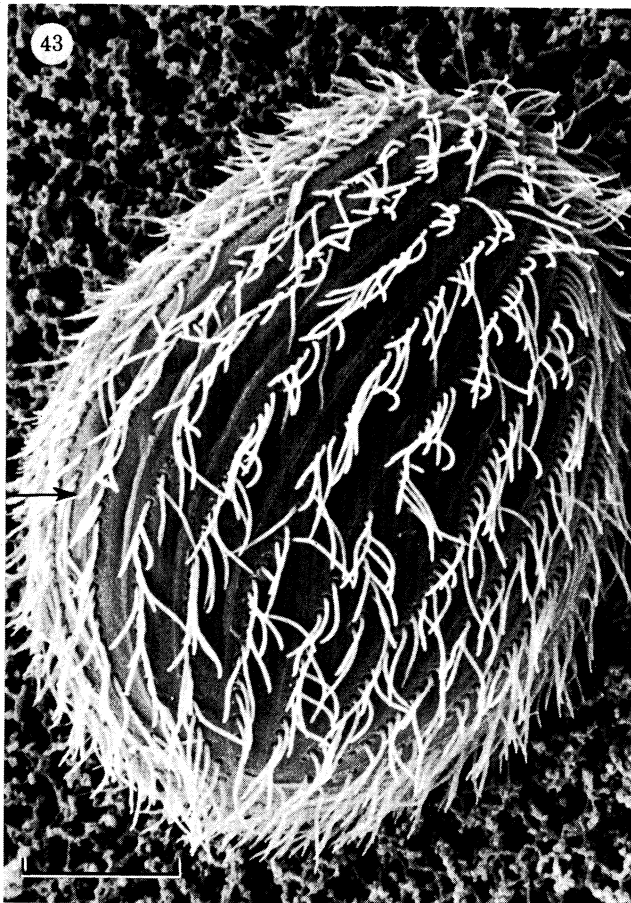
The number and arrangement of the somatic kineties are of particular interest. Not only does the total number of kineties span an extraordinarily wide range, from approximately 30 to approximately 66, but perhaps more significantly they are subject to degrees of disorganization ranging from slight to severe (figures 4–9). The following primary categories of deviation have been observed: truncated kinety, branched kinety, fragmented kinety, meandering kinety, irregular kinetosome spacing, isolated kinetosomes (figure 4). Some individuals display a relatively ordered cortex, exhibiting only one or two of the above deviations in perhaps only a few kineties (figure 5). Other ciliates, from the same infected larva, manifest all categories of deviation with few kineties unaffected (figures 4, 6, 7). Many cells lie in between these two extremes (figures 8, 9). In some instances cortical disorganization is locally so severe that individual kineties cannot be distinguished (figure 7). According to their severity these deviations lead to greater or lesser difficulties in accurate quantification of the total number of somatic kineties and post-oral kineties (figures 8, 10), and in specifying the location of superficial structures normally defined in relation to the somatic kineties, such as contractile vacuole pores.

Although not always conspicuous, fine argentophilic structures corresponding in general appearance to the secondary meridians of other tetrahymenids occur between the somatic kineties of *T. dimorpha* recovered from larval hosts (figures 11, 12). Like the ciliated meridians they often lack order, sometimes producing complex looped and twisted configurations which frequently make direct contact with the somatic kineties (figures 11, 12).

In general the kinetosomes are packed in a remarkably dense fashion along the kineties (figures 10–12), often so densely that the discrete areas of silver that indicate the position of each kinetosome appear to abut one another (figures 10, 11). That many of these kinetosomes do bear cilia is revealed by scanning electron microscopy. Indeed parasites from larval hosts are so heavily clad in cilia that it is often difficult to see details of the underlying cell surface (figure 15). Where the cell surface and points of ciliary insertion are visible, it is clear that many of the displaced kinetosomes belonging to disorganized kineties do indeed bear cilia (figure 16). Nomarski interference contrast observations upon living ciliates confirmed both that the cell is very densely clad in cilia, and also that all categories of kinetal disorganization could be identified from the spatial arrangement of cilia.

The intensely hairy appearance (dense somatic ciliature) and tendency towards deformity (disorganized cortex) of ciliates recovered from larval hosts prompts the author to designate this distinctive morphological form the 'Hyde' type (Stevenson 1886). Further justification for the use of this term will become clear in later sections.

DESCRIPTION OF PLATE 7

Scanning electron micrographs of *T. dimorpha* sp.nov. cultured in Mitsuhashi and Maramorosch insect tissue culture medium supplemented with 20 % foetal calf serum.

FIGURE 43. Hyde cell displaying a variety of kinetal abnormalities, including branching (central region) and a single truncated kinety (arrow). Scale bar, 10 μm.

FIGURE 44. Detail of cortical surface, showing precise ciliary dispositions. Branches of various lengths are clearly visible (arrows), as is a single displaced cilium (c.), which may represent the first step in the formation of a branch. Wide gaps are often associated with kinetal branching. Scale bar, 5 μm.

FIGURE 45. Part of cortex of de-ciliated Hyde cell, giving an uncluttered view of kinetal disarray. Scale bar, 5 μm.

FIGURE 46. Posterior region of Hyde cell bearing two contractile vacuole pores in normal positions plus an additional contractile vacuole pore in an abnormal interkinetal position (arrow) approximately halfway towards the cell equator. Scale bar, 5 μm.

TABLE 2. MORPHOMETRIC DATA OF SILVER STAINED *TETRAHYMENA DIMORPHA* SP.NOV. FROM *IN VIVO* AND *IN VITRO*

	total number of somatic kineties				somatic length				somatic width				ratio mean somatic length: mean somatic width
	mean μm	s.d. μm	range μm	n	mean μm	s.d. μm	range μm	n	mean μm	s.d. μm	range μm	n	
Hyde cells from larval host	50.5	7.4	30–66†	40	68.9	7.3	55.9–89.4	50	50.7	6.1	41.3–70.4	50	1.36:1
Jekyll cells from adult host	21.0	0.6	19–22	50	42.0	3.3	33.5–49.2	50	28.6	2.5	24.6–37.5	50	1.46:1
Jekyll cells from bacterized rice infusion	21.3	0.7	20–24	40	52.2	4.8	41.3–65.9	40	26.0	3.1	19.0–33.0	40	2.01:1
Hyde cells from dimorphic p.p.y.e. culture	33.9	4.2	25–44†	40	67.6	6.6	51.4–81.6	40	50.1	6.5	36.9–63.7	40	1.35:1
Jekyll cells from dimorphic p.p.y.e. culture	22.2	2.0	19–26	40	36.4	2.4	32.4–41.3	40	19.1	1.7	16.8–23.5	40	1.91:1
Hyde cells from supplemented M. & M. culture	27.8	3.0	23–39†	40	64.8	6.4	54.3–80.0	40	47.9	6.4	34.3–60.0	40	1.35:1

	length of membranelle 1				length of membranelle 2				length of membranelle 3			
	mean μm	s.d. μm	range μm	n	mean μm	s.d. μm	range μm	n	mean μm	s.d. μm	range μm	n
Hyde cells from larval host	6.3	0.64	5.6–7.3	17	5.4	0.58	4.5–6.2	17	4.8	0.44	4.5–5.6	17
Jekyll cells from adult host	6.4	0.29	6.2–6.7	20	5.5	0.12	5.0–5.6	20	4.5	0.31	3.9–5.0	20
Jekyll cells from bacterized rice infusion	7.6	0.46	6.2–8.4	40	6.6	0.37	6.2–7.3	40	5.4	0.31	4.5–6.2	40
Hyde cells from dimorphic p.p.y.e. culture	7.2	0.56	6.2–8.4	20	6.4	0.58	5.6–7.3	20	5.6	0.54	4.5–6.7	20
Jekyll cells from dimorphic p.p.y.e. culture	6.6	0.31	6.2–7.3	30	5.8	0.32	5.0–6.2	30	4.9	0.41	4.5–5.6	30
Hyde cells from supplemented M. & M. culture	7.3	0.52	6.7–8.4	25	6.5	0.47	6.2–7.3	25	5.7	0.24	5.6–6.2	25

Abbreviations: s.d., standard deviation; p.p.y.e., proteose–peptone–yeast-extract; M. & M., Mitsuhashi and Maramorosch insect tissue culture medium.
† Approximate values, owing to difficulties imposed by cortical disorganization.

Notwithstanding the difficulties created by kinetal disorganization, ciliates from larval hosts most commonly possessed two to four post-oral kineties, though as few as one or as many as seven were sometimes recorded (mean value 3.29 ± 1.46, $n = 27$). Normally one or two contractile vacuole pores were present (figure 13); occasionally there were three or rarely four (mean 1.65 ± 0.72, $n = 43$). Contractile vacuole pores were associated with the posterior termini of kineties 5–9, irrespective of the total number of somatic kineties present. Rarely contractile vacuole pores were located interkinetally in an equatorial position (figure 14), but still within the normal longitudinal zone, i.e. the 5–9 kinetal band. Such cells bearing equatorial contractile vacuole pores were not involved in stomatogenesis, nor did any other features indicate imminent cell division.

Anteriorly up to 20 kineties converge to form a pre-oral suture, the remaining kineties terminating short of the cell apex so delineating a 'bald' anterior pole (figures 8, 9).

Stomatogenesis was observed only very rarely in ciliates from larval hosts, the stomatogenic kinety being number 1. A cytoproct could not be detected.

Nuclear characteristics

Feulgen-stained preparations revealed that in air-dried preparations the macronucleus is an irregular oval structure measuring 28.3 ± 4.6 μm (19.0–36.9 μm, $n = 23$) $\times 18.6 \pm 3.8$ μm (12.3–27.9 μm, $n = 23$). The spherical or almost spherical diminutive micronucleus was generally located in close association with the macronucleus and measured 1.6 ± 0.2 μm in diameter (1.2–2.0 μm, $n = 26$). Macronuclear chromatin extrusion bodies were present in approximately 24% ($n = 253$) of ciliates from larval hosts. Macronuclear chromatin extrusion bodies measured up to 10 μm in diameter and stained noticeably more intensely than either the macro- or micronucleus. Only rarely did a single cell contain more than one macronuclear chromatin extrusion body.

Parasites in pupal and adult hosts

At pupation or shortly thereafter the size of the population of parasitic ciliates in the host's haemocoel began to increase significantly. In the laboratory, within 36–60 h of pupation at 15 °C the number of ciliates had increased from the mean larval level of 147 to a mean approximate value of 640 (450–850, $n = 7$). This trend continued so that at eclosion, which at 15 °C occurs at about 9 days after pupation, a single fly might contain an estimated 5500–9000 ciliates ($n = 5$). Even with this elevated parasite burden recently emerged infected adults did not exhibit any overt behavioural or morphological abnormality and their capacity for flight seemed unimpaired. However, increasing morbidity soon became apparent and death of the adult generally occurred within 2–4 days post-eclosion at 15 °C, by which time an estimated 11 000–19 000 ciliates were present in the host's haemocoel. Careful dissection of infected adults showed that the haemocoel was densely packed with ciliates, and that only tiny residual islands of fat body remained, but no detailed histological study was undertaken, and so the precise histopathological details and the cause of host death remain uncertain.

Both sexes of *S. equinum* are susceptible to infection with *T. dimorpha*. Of eleven infected adults, the host stage most easily and reliably sexed, seven were male and four were female.

The acceleration of ciliate population growth that accompanies metamorphosis of the host from larval to pupal–adult phases is further attended by a startling morphological transformation of the ciliates. This transformation is manifest in changes in cell size, cell shape and particularly in cortical organization.

Data concerning cell length and cell width of ciliates recovered from adult hosts are given in table 2. Based upon that data, mean cell volumes were calculated by using the formula $\frac{4}{3}\pi xy^2$, where x and y are the major and minor semi-axes of the cell (Seaman *et al.* 1972). Values of 92719 and 18034 μm^3 were obtained for parasites from larval and adult hosts respectively, a ratio of 5.14:1.

The broadly oval form of ciliates recovered from larval hosts gradually gave way to a more elongate or pear-shaped form (figures 17, 18). This change is reflected in a slight increase in the somatic length:somatic width ratio (table 2). Living ciliates from adult hosts retain the cytoplasmic granularity that imparts opacity in transmitted light (figure 19). In contrast to ciliates recovered from larval hosts, many of those recovered from pupal and adult hosts were undergoing stomatogenesis or exhibited other signs of recent or imminent cell division.

Cortical features

Morphometric features are summarized in table 2. The most significant changes relate to the somatic kineties; not only was there a remarkable 60% decrease in the mean total number of somatic kineties from over 50 to 21, but furthermore this quantitative change was accompanied by the establishment of typically tetrahymenine orderliness (figures 17, 18). The somatic kineties lost the disorganized arrangement so characteristic of ciliates from larval hosts. Only occasional vestiges of the previous cortical disarray were apparent, some cells possessing one or sometimes two incomplete kineties (figure 18); other categories of kinety disorder were not apparent. The fine argentophilic secondary meridians also became very ordered in their arrangement, running midway between and parallel to the ciliated meridians (figure 17).

In general, kinetosomes of the somatic kineties were much less densely packed in ciliates from adult hosts than in those from larval hosts. Together with the dramatic reduction in total somatic kineties, this produced cells that were much more sparsely clad in cilia (figure 18). Comparison of silver-stained preparations from larval and adult hosts suggests that the total kinetosome population in each cell type might be significantly different. In a random sample of non-dividing cells from each source the total number of kinetosomes in the region of kineties $n-2$ to $n-5$ was counted. From mean total somatic kinety data, a mean total kinetosome count could be calculated. Values of 4290 ($n = 7$) and 720 ($n = 11$) were obtained for ciliates from larval and adult hosts respectively, a ratio of 6:1.

Dimensions of oral apparatus components remained similar to those in ciliates from larval hosts (table 2), but owing to the reduction of somatic dimensions the relative size of the oral apparatus increased (cf. figures 8, 17). One or sometimes two short pre-oral kinetal segments were seen in many specimens (figure 17).

A general reduction in hairiness (less dense somatic ciliature) and distinct assertion of orderliness (highly organized cortex) of ciliates recovered from adult hosts prompts the author to name this morphological form the 'Jekyll' type (Stevenson 1886).

Nuclear characteristics

The macronucleus is significantly smaller than that of ciliates from larvae. Feulgen-stained macronuclei measured 16.0 ± 4.2 μm ($n = 20$) by 10.6 ± 2.6 μm ($n = 20$). The diameter of the micronucleus remained unchanged at approximately 1.5 μm. Approximately 86% of cells contained macronuclear chromatin extrusion bodies ($n = 147$) and often more than one per cell was noted. Conjugation of ciliates in adult hosts, or nuclear profiles that might have suggested recent conjugation, were never observed.

Free-living ciliates

Ciliates derived from infected larval, pupal and adult hosts were successfully established in bacterized infusions based upon rice or wheatgerm in Prescott and James solution. It was possible to maintain such cultures for many months by periodic subcultivation. Upon release into Prescott and James solution from the host's haemocoel a proportion of ciliates died owing to ionic–osmotic shock. The proportion was very variable but in general the more mature the host, the less mortality was incurred. Ciliates from larval hosts were particularly susceptible to ionic–osmotic shock, and 100% mortality was common at 15 °C. Reduction in mortality was obtained by lowering to 10 or 5 °C the temperature at which ciliates were released from the host. When ciliates were released from any host stage they underwent characteristic swelling as a result of ionic–osmotic changes (figure 19). The appearance of lytic blebs as the cell membrane lifted away from the surface preceded sudden, sometimes explosive, rupture of the cell, which marked its death. Surviving cells did not develop such lytic blebs, and over a period of several hours they lost the swollen almost spherical aspect of osmotic–ionic stress and regained their normal proportions. Following this period of re-adjustment, which at 15 °C lasted for up to 18 h, feeding and cell division began (figure 20). During the course of the first few cell divisions free of the host the cytoplasmic granularity which characterized parasitic ciliates was gradually lost (figures 19–21). The accompanying tendency towards a more elongate cell shape is reflected in the changing ratio of somatic dimensions (table 2). The precise form of ciliates varied, those in freshly produced infusions being more portly (figure 22) than those in older cultures (figure 23). The cytoplasm of ciliates in infusion was generally packed with food vacuoles (figures 22, 23).

Cortical features

Morphometric data from silver-stained specimens are summarized in table 2. The cortex of ciliates from infusions resembled closely that of ciliates from adult hosts. The cortex was very highly ordered, exhibiting not even the residual disorganization sometimes seen in parasites from adult hosts. The ciliated primary and non-ciliated secondary meridians ran from pole to pole, except where interrupted by the oral apparatus (figures 24–28). The mean total number of somatic kinetics, 21.3, was not significantly different from that of ciliates from adult hosts (table 2). One or rarely two short pre-oral kinetal segments, each comprising four to eight kinetosomes, occurred anterior to the oral apparatus in approximately 90% of specimens (figure 29). The number of post-oral kineties varied from one to three, with approximately 75% of all specimens having two ($n = 46$) (figures 25, 29). Only a single contractile vacuole pore was ever observed, and this always occurred in association with kinety 5 or 6 (figures 26, 27). A cytoproct was situated between kineties 1 and n towards the posterior pole (figure 27). The stomatogenic kinety was always number 1 (figure 25).

Nuclear characteristics

The size of the macronucleus in air-dried, Feulgen-stained smears of ciliates from infusions was reduced to $8.0 \pm 1.3 \ \mu m \times 5.7 \pm 1.1 \ \mu m$ ($n = 25$). The diameter of the micronucleus remained unchanged at approximately 1.5 μm. Macronuclear chromatin extrusion bodies were observed only rarely. Evidence of conjugation was never obtained.

In vitro *cultivation of ciliates*

Proteose–peptone–yeast-extract medium

Ciliates isolated axenically from the haemocoel of larval, pupal and adult *S. equinum* developed into dimorphic populations in proteose–peptone–yeast-extract medium, with two distinct morphological forms present, both Jekyll and Hyde (figure 30). The two forms remained clearly distinct in size, shape and cortical organization as revealed by silver-staining (table 2). Usually the dimorphic nature of the population became apparent within 2–5 days of initiation of the culture, irrespective of the origin (and hence the morphological form) of the ciliate inoculum. This time scale was not invariably the case however; in one experiment, for example, four isolates, each of approximately ten ciliates from the same infected adult male host, exhibited overt dimorphism at 11, 63, 70 and 84 days post-isolation respectively.

Once dimorphism was established in a proteose–peptone–yeast-extract culture, the relative proportions of Jekyll and Hyde cells varied over the course of many months from approximately 1:1 to 1:50. Most commonly Hyde cells were outnumbered by Jekyll cells by about 10:1. The oldest cell lines have been maintained for over 2 years with no apparent loss of vigour, or loss of dimorphic expression.

Most cell lines were established from initial isolates comprising more than one ciliate, but even those established from single ciliates manifested concurrent dimorphism in sterile proteose–peptone–yeast-extract culture. Nevertheless a series of cloning experiments was done to eliminate absolutely the possibility that the apparent dimorphism (both *in vitro* and *in vivo*) was due, for example, to the presence of two strains or two species of ciliate. Cloning of Jekyll and Hyde cells from proteose–peptone–yeast-extract cultures always produced dimorphic cultures. Usually dimorphism was apparent within 2–5 days, but sometimes up to 3–5 weeks was required. A total of 50 cells, five Jekyll and five Hyde from each of five cell lines (each line initiated from a different original host), were cloned in proteose–peptone–yeast-extract and all cells behaved consistently in the production of dimorphic populations (figure 30).

The somatic dimensions of Hyde and Jekyll cells grown in proteose–peptone–yeast-extract differed significantly from each other (table 2). By using the same formula as before, mean cell volumes of 88 860 and 6970 µm³ were calculated for Hyde and Jekyll cells respectively, a ratio of 12.75:1.

Cortical features

Although their overall somatic dimensions were smaller, the general body form and the organization of the cortex of Jekyll cells grown in proteose–peptone–yeast-extract corresponded very closely, both qualitatively and quantitatively, with Jekyll cells from adult hosts and with those from infusions (table 2, figures 30, 31, 39).

Similarly, Hyde cells from the same medium displayed many of the features characteristic of those ciliates recovered from larval hosts. Indeed the overall shape and size of the cells were almost identical (table 2, figure 30). It should be noted that, although both the range and the mean total somatic kinety number were substantially lower than those of Hyde cells from larval hosts, nevertheless the characteristic cortical disorganization was clear (figures 33–36). All categories of kinetal disarray were apparent and there was once again a great variation between individual ciliates in the degree to which cortical disorganization was displayed. Hyde cells in proteose–peptone–yeast-extract medium displayed the same closely packed somatic kinetosomes

(figures 33–36) and dense somatic ciliature (figure 38) as those from larval hosts. Normally one or two contractile vacuole pores were present in association with the posterior ends of kineties 4 to 7, but, as in Hyde cells from larval hosts, abnormally positioned contractile vacuole pores were sometimes seen. Typically two to four post-oral kineties were present. Although normally kinety number 1 was stomatogenic, sometimes kinety n was. Homopolar doublets were sometimes seen and also oral replacement.

Almost all cells in proteose–peptone–yeast-extract culture fell clearly into one or other of the two distinct morphological types. A very small proportion of cells ($< 1\%$) displayed characteristics intermediate between the two forms (figure 37).

Nuclear characteristics and sexuality

The micronuclear diameter of both Jekyll and Hyde cells remained unchanged at approximately 1.5 μm. Macronuclear dimensions were in accordance with the size of the cell, measuring approximately 18 μm × 14 μm in Hyde cells and 9 μm × 6 μm in Jekyll cells.

Conjugation in *T. dimorpha* was never observed in infected larval, pupal or adult hosts, or in infusions. In sterile culture proteose–peptone–yeast-extract was the only medium in which conjugation was ever seen to take place. Conjugation was recorded in a single cell line originally derived from an infected adult male *S. equinum*. By day 10 following initiation of this cell line the population was typically dimorphic and approximately 10–12% of the Jekyll cells were involved in conjugation (figure 32); Hyde cells were never seen to conjugate. A declining incidence of conjugation in this cell line was observed during the following 12 weeks; thereafter no further conjugation was seen.

Insect tissue culture media

Ciliates grown in Mitsuhashi and Maramorosch insect tissue culture medium supplemented with 20% foetal calf serum exhibited a morphology characteristic of Hyde cells (figure 40). Cell shape and somatic dimensions were very similar to Hyde cells from larval hosts or from proteose–peptone–yeast-extract culture (table 2). The mean total number of somatic kineties was lower and the range narrower than that found in Hyde cells from other sources (table 2). Qualitatively, however, the cortex manifested the same deviations as before (figure 41, 42). Indeed Hyde cells grown in serum-supplemented Mitsuhashi and Maramorosch insect tissue culture medium illustrate particularly well in scanning electron microscopy the cortical disorganization revealed by silver-staining in Hyde cells from other sources (figures 43, 44). This was due to the presence of fewer kineties in cells of undiminished size. Furthermore some cells became partially or wholly deciliated during the preparatory process, and the cortex of such cells provided an uncluttered view of kinetal disarray (figure 45). Abnormal contractile vacuole pore positions were also readily seen by scanning electron microscopy (figure 46).

Ciliates were found to grow only very slowly in unsupplemented Mitsuhashi and Maramorosch insect tissue culture medium and eventually such cultures died despite regular subcultivation. Grace's insect tissue culture medium with or without haemolymph, foetal calf serum or newborn calf serum supplements was unsuccessful for *T. dimorpha*. The ciliates survived with very little population growth and with the appearance of grossly aberrant forms for only a few weeks before dying.

SYSTEMATIC ACCOUNT

Tetrahymena dimorpha sp.nov. (figures 1–46, table 2)

Diagnosis. Dimorphic, the two distinct morphological forms occur naturally during the life cycle and are named Hyde and Jekyll after another strange case of dimorphism (Stevenson 1886). Both forms may be cultivated *in vitro*.

Hyde form. Parasitic in larval simuliids; broadly oval with dense granular cytoplasm; uni-micronucleate; somatic dimensions approximately 69 μm × 51 μm; very dense somatic ciliature; mean total number of somatic kineties approximately 50, with a wide range from 30 to 66; few to many of the kineties are characteristically abnormal (branched, incomplete, meandering, etc.); one to seven post-oral kineties, usually two to four; one to four contractile vacuole pores, associated with kineties 5–9; conspicuous pre-oral suture; oral apparatus typically tetrahymenine.

Jekyll form. Parasitic in adult simuliids and free-living in the laboratory; presumed natural free-living form; more elongate shape; free-living cells without densely granular cytoplasm; uni-micronucleate; somatic dimensions approximately 42 μm × 29 μm in adult simuliids and 52 μm × 26 μm when free-living in infusion; relatively sparse somatic ciliature; mean total number of somatic kineties approximately 21 with narrow range of 19–24; somatic kineties highly ordered and without abnormalities; one to three post-oral kineties, usually two; single contractile vacuole pore associated with kinety 5 or 6; oral apparatus typically tetrahymenine and of similar dimensions to that of the Hyde form.

Differentiating characters. Unique dimorphism; Jekyll cells bear strong resemblance to members of the *T. pyriformis* complex.

Type host. Larval, pupal and adult stages of *Simulium equinum* (Diptera: Simuliidae); the ciliate also occurs, though very rarely, in *S. ornatum*.

Host tissue involved. Ciliates occur free in the haemocoel in all stages of the host.

Type locality. River Wey, near Tilford, Surrey, U.K.

Derivation of name. Dimorpha after its distinctive dimorphism.

Type specimens. In view of the dimorphism displayed by *T. dimorpha* it is not possible to designate a single preparation as holotype. Consequently, and in accordance with the recommendations of Corliss (1972a), a series of three slides are hereby designated as the holotype for *T. dimorpha*. These three slides have been deposited in the British Museum (Natural History), London and comprise the following silver-stained preparations: parasitic ciliates from haemocoel of larval *S. equinum* (slide no. 1982:12:16:1); free-living ciliates from bacterized rice infusion (slide no. 1982:12:16:2); dimorphic population of ciliates from axenic proteose–peptone–yeast-extract culture (slide no. 1982:12:16:3). Paratype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington D.C., U.S.A., and comprise the following silver-stained preparations: parasitic ciliates from haemocoel of larval *S. equinum* (slide no. U.S.N.M. 32216); free-living ciliates from bacterized rice infusion (slide no. U.S.N.M. 32217); dimorphic population of ciliates from axenic proteose–peptone–yeast-extract culture (slide no. U.S.N.M. 32218). Further paratype slides are held in the collections of John Corliss (Department of Zoology, University of Maryland, U.S.A.) and of the author.

DISCUSSION

Relating to taxonomy and the current status of ciliatoses in Simuliidae

Features of the Jekyll cells clearly indicate that the new species fits well into the genus *Tetrahymena*. Cell size, shape, arrangement and number of somatic kineties, structure of the oral apparatus, position and number of contractile vacuole pores, post-oral kineties and cytoproct, all are in accordance with the generic characteristics of *Tetrahymena* (Corliss 1973). A facultative or obligate parasitic habit is shared by some other members of the genus, but it is the nature of the remarkable dimorphism associated with the parasitic habit of *T. dimorpha* sp.nov. that sets it apart from other species.

A number of di- or polymorphic species already exist in *Tetrahymena*, notably the free-living species *T. patula*, *T. vorax* and *T. paravorax* which possess both macrostome and microstome forms. Even the intensively studied *T. thermophila* only recently has been found to manifest dimorphism under certain conditions (Nelsen & Debault 1978). Among those species that exhibit parasitism, only *T. rostrata* and *T. limacis* are di- or polymorphic. One characteristic that separates the morphological types in these species is the number of somatic kineties, which has a bimodal distribution; parasitic forms possess significantly more somatic kineties than do free-living stages (Corliss 1973). A similar, although more extreme, bimodality is expressed in the two morphological forms of *T. dimorpha*.

Corliss (1973) has pointed out the importance of biological properties other than morphology in the reliable differentiation of ciliate species. Physiological, biochemical and ecological data are of great value in this context. In ciliates that exhibit parasitism, the nature of the host–parasite relationship is important in species designation. The relationship between *T. dimorpha* and its simuliid host is unusual among known insect-parasitic ciliates in the benign nature of the infection in larval hosts and its persistence into the adult. Whatever the mechanism that appears to limit the ciliate population within the larval host, the end result is an infection that does not interfere with host pupation or eclosion. Only in the adult fly itself does the infection become pathogenic, a situation that contrasts markedly with other ciliate infections of insects (Corliss 1960; Barthelmes 1960; Clark & Brandl 1976). The number of *T. dimorpha* in larval *S. equinum* increases very slowly, and cell division is rarely seen. Overwintering *S. equinum* larvae require up to 6 months for completion of the larval phase. Even with the assumption that each host is initially infected by a single ciliate early in larval development, a mean cell-cycle time up to 23 days would be required in order not to exceed the maximum observed larval burden. Low ambient water temperatures may contribute to the generation of long cell-cycle time. However, infected larvae of the summer generations of *S. equinum* develop in river water having a mean temperature of 12–16 °C, and yet the number of ciliates per host remains within the same limits. *In vitro* at 15 °C a cell-cycle time of approximately 20 h has been obtained for Hyde cells in serum-supplemented insect tissue culture medium (unpublished observations). On that basis an infected larva containing ten ciliates would in just 1 week produce over 2500 ciliates at 15 °C; this clearly does not happen in the field, nor in infected larvae reared in the laboratory for up to 2 weeks at that temperature. It is possible that some specific nutritional or hormonal function of the larval haemocoel may be responsible for direct limitation of the ciliate population. Both nutritional and hormonal profiles are known to be subject to changes during metamorphosis (Riddiford 1980) and might be responsible for the enhanced ciliate population

growth (and perhaps the concurrent morphological transformation) which occurs during pupal and adult phases of the host life cycle.

Tetrahymena dimorpha from all infected stages of the host are able to become free-living and, since the available evidence does not support transovarial transmission of the parasite, it seems probable that free-living *T. dimorpha* are responsible for re-infection of successive generations of simuliid larvae. Laboratory attempts to infect *S. equinum* with *T. dimorpha* have so far been unsuccessful, and naturally infected hosts yield no clues regarding the precise mode of infection. Indeed scant information is available concerning the route of infection in other cases of insect ciliatosis (Corliss 1960, 1973), although two main routes of direct infection may be postulated: across the gut (via mouth or anus) or through the cuticle (by active penetration, or through natural breaks especially during ecdysis). In only a single insect-parasitic ciliate, *Lambornella clarki*, is the route of infection well documented (Corliss & Coats 1976); in that ciliate a cuticular penetration cyst is produced. Further data regarding the ecology of *T. dimorpha* are necessary before the precise nature of its relationship with *S. equinum* can be satisfactorily established. It is not merely semantic argument to raise the question: is *T. dimorpha* a facultatively free-living parasite or a facultatively parasitic free-living ciliate? The specificity and apparent subtlety of the association, together with its temporal consistency, are contrary to the idea that *T. dimorpha* is simply an opportunistic parasite. Nevertheless it would be valuable from many viewpoints, including the evolutionary one (Corliss 1972b), to understand more clearly the relative obligacy of different phases of its life cycle.

The occurrence of ciliates as parasites in the haemocoel of Simuliidae has been sporadically recorded (Vargas 1945; Lewis 1952, 1960a, b; Marr & Lewis 1964), mainly from Africa. During the past 5 years the author has observed ciliates, which he suspects belong to *Tetrahymena*, in larval simuliids in New Zealand and southwest Germany (unpublished observations), but at an incidence and intensity of infection too low to permit serious study. The first clear record of *Tetrahymena* in Simuliidae was made by Corliss *et al.* (1979). They briefly described the occurrence of *Tetrahymena* sp. in the haemocoel of adult male and female *Simulium damnosum* from the Ivory Coast. More recently *Tetrahymena rotunda* has been described from *S. tuberosum* and *S. venustum* in North America (Lynn *et al.* 1981). *T. rotunda* was recorded from larval, pupal and adult stages of the host, but quantitative changes of the parasite population within these stages were not documented, nor was there any suggestion of morphological differences between parasites from different host stages. Common features do exist, however, between *T. rotunda* and the Hyde form of *T. dimorpha*, for example the general size, shape and mean total number of somatic kineties. There are also significant differences; although *T. rotunda* possesses intercalated kineties (also found in some other parasitic tetrahymenids) it does not manifest the range of gross kinetal disorganization so characteristic of the Hyde form of *T. dimorpha*. Additionally, the range of total somatic kineties and of post-oral kineties is significantly greater in *T. dimorpha*. Contractile vacuole pores were not recorded in silver-stained specimens of *T. rotunda*, whereas they are a consistent feature of *T. dimorpha*. In contrast to *T. dimorpha*, *T. rotunda* could not be cultivated in media suitable for other tetrahymenids (Lynn *et al.* 1981). Most significantly, *T. rotunda* has so far not been observed to manifest overt dimorphism. It should be noted, however, that most of the data for *T. rotunda* were derived from larval infections. During a 5 year period only three infected pupae and one infected adult were studied. It is possible therefore that morphological changes in the parasites of pupal and adult hosts might perhaps have been overlooked. *Tetrahymena rotunda* needs to be re-examined with possible

dimorphism in mind. This need is emphasized by observations that suggest that the *Tetrahymena* sp. recovered from *S. damnosum* adults (Corliss *et al.* 1979) is more typically tetrahymenine and bears superficial resemblance to the Jekyll form of *T. dimorpha*. In view of the scant data regarding these other species parasitizing simuliids, the possibility that they may exhibit dimorphism cannot be discounted. Indeed, it is tempting to speculate that there exists a group of closely related dimorphic *Tetrahymena* species infecting simuliids in geographically widespread locations (*T. dimorpha*, U.K.; *T. rotunda*, North America; *Tetrahymena* sp., west Africa; suspected *Tetrahymena* spp., New Zealand and Federal Republic of Germany). In the meantime the ciliate that is the subject of this paper remains clearly distinct from other ciliates infecting simuliids and from the remaining members of the genus, and a new species *Tetrahymena dimorpha* is proposed.

Relating to morphogenesis

Dimorphism in *Tetrahymena dimorpha* is apparent as an integral part of the natural life cycle of this ciliate and it is also expressed *in vitro* by the application of certain axenic and non-axenic culture conditions. In its manifestation of such distinct and controllable dimorphism this new member of the genus *Tetrahymena* provides a valuable model for the study of certain aspects of ciliate morphogenesis.

Studies of cortical inheritance in ciliates clearly demonstrate that existing structures in the cortex play an important role in determining the organization of new structures, a process that has been termed cytotaxis (Sonneborn 1964) or structural inertia (Nanney 1980). Implicit in the concept of cytotaxis is the high degree of autonomy apparent in ciliate cortical patterning (Aufderheide *et al.* 1980). Cytotaxis is not, however, an absolutely rigid phenomenon, as demonstrated by corticotype variation within ciliate clones (Nanney 1966*a*, *b*) and in this study. Furthermore it is becoming increasingly clear that genic factors are also involved in the determination of cortical structure, not only in specifying the molecular components of cortical units but also in the specification of cortical pattern itself. Heckmann & Frankel (1968) have provided evidence that long-term genic control of corticotype is superimposed upon the cytotactic mechanism in *Euplotes minuta* and is ultimately responsible for maintenance of the most stable corticotype. Frankel (1973) has further demonstrated that an abnormality in the proliferation and spatial disposition of basal bodies in *Euplotes* is genically determined and can undermine the fidelity of cytotaxis.

A combination of genic and cytotactic control mechanisms seems therefore to be responsible for the determination of cortical phenotype in ciliates (Aufderheide *et al.* 1980), and further study of the relative contributions of these two mechanisms is possible through the use of *T. dimorpha*. Jekyll cells, with their well ordered kineties lying within a narrow and stable range, exhibit features consistent with the traditional concept of cytotaxis; Hyde cells do not.

Abnormalities in the configuration of *Tetrahymena* kineties have been noted previously in a variety of 'monstrous' forms generated by exposure of normal cells to heat shock, to flattening on agar or gelatin plates, to high pressure or to viscous solutions of methyl cellulose (Hjelm 1977; Frankel 1964; Rosenbaum *et al.* 1966; Simpson & Williams 1970). Kinetal abnormalities are also apparent after treatment of normal ciliates with mutagens (Sonneborn 1974) and even in untreated cultures (Nanney 1959). Organization of the cortex of *T. dimorpha* is remarkable in so far as kinetal abnormalities are apparent as a normal feature of the natural life cycle of the ciliate and *in vitro*.

Other unusual features of the cortical dimorphism displayed by *T. dimorpha* are mentioned

briefly here as a further indication of the possible value of this ciliate in morphogenetic studies. Nanney & Chow (1974) demonstrated the phenomenon of basal body homeostasis in members of the *T. pyriformis* complex. This principle, which implies a high degree of interdependence of kinetosome proliferation and cell fission, clearly does not operate during transformation of one *T. dimorpha* phenotype to the other, as shown by the 6:1 ratio of mean total kinetosomes (Hyde:Jekyll). Specification of contractile vacuole pore longitude in *T. dimorpha* may not be on the same basis as in members of the *T. pyriformis* complex. Normally contractile vacuole pore meridians bear a fixed spatial relation to the oral apparatus meridian, a relation defined by a so-called inductive angle, so that the actual contractile vacuole pore meridian number changes with increasing corticotype. In the Jekyll and Hyde cells of *T. dimorpha* contractile vacuole pores are formed within the region delineated by kineties 5–9, irrespective of the total number of kineties present, whether 20 or 60.

One of the outstanding features of most ciliates is the precision of events leading up to the formation of identical individuals at fission. *Tetrahymena dimorpha* is an exception to this rule and the fact that its unique properties are expressed under normal physiological conditions is a measure of its potential value in studies of ciliate morphogenesis.

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