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Comparison of the Esterases and Acid Phosphatases in *Paramecium multimicronucleatum*, Syngens 1-5, *P. jenningsi*, *P. caudatum*, and the *P. aurelia* Complex[1]

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ABSTRACT. Forty-eight stocks in *Paramecium jenningsi*, syngens 1–5 of *P. multimicronucleatum*, *P. caudatum*, *P. primaurelia*, *P. biaurelia*, and *P. tetraurelia* were grown axenically and tested for their esterases and acid phosphatases using starch gel electrophoresis. The five esterases and the acid phosphatases previously characterized in species of the *P. aurelia* complex were also found in *P. jenningsi*, and three to four of the esterases and the acid phosphatases were found in the *P. multimicronucleatum* species complex and in *P. caudatum*. Additional subtypes were observed for each of the enzyme phenotypes in these new (though here unnamed) species of *Paramecium*. Two of the new acid phosphatase subtypes, which depart radically in mobility and in pattern, were found in syngen 3 of *P. multimicronucleatum* and in *P. caudatum*. Except for syngens 1 and 5 in *P. multimicronucleatum*, the degree of similarity between syngens 1, 5 and 2, 3, and 4 appears to be very low—perhaps even lower than that seen for species in the *aurelia* complex. More realistically, the syngens of *P. multimicronucleatum* should be considered as separate species although they are not here given separate taxonomic names. Limited sharing of subtypes occurred between species in different species complexes. This observation suggests that the molecular distances between species complexes may be even greater than between species within a complex.

SPECIES relationships in the *Paramecium aurelia* complex have been probed using the technique of enzyme electrophoresis: all 14 species can be distinguished on the basis of phenotypic differences observed for several different enzymes (4, 6, 7, 25, 26). Interspecies comparisons have led to estimation of similarity coefficients and/or genetic distances between the species of the *aurelia* complex (1, 4, 26) and to the construction of dendrograms that can be interpreted as representing the evolution of this group of species (1, 4). These comparisons showed that similarity coefficients are usually low and the distances between species large. One of these studies, which included stock 8MO in syngen 3 of *P. multimicronucleatum*, concluded that there was no clear-cut distinction between it and the species of the *aurelia* complex (1).

Other species complexes also occur in *Paramecium*, including *P. multimicronucleatum* and *P. caudatum*. These species, along with *P. jenningsi*, all have a body shape similar to the *P. aurelia* species, but they differ in size and in nuclear number or morphology (20). Originally, *P. jenningsi* and the first three syngens of *P. multimicronucleatum* were classified as syngens of *P. aurelia* (20, 21). The "complex" *P. aurelia-multimicronucleatum* referred to "paramecia with vesicular micronuclei and cigar-shaped bodies"; however, it was noted that this complex was divisible into three groups on the basis of micronuclear characteristics (24). Group 1 had two micronuclei of intermediate size with the chromatic areas in the form of a doughnut; group 2 had four micronuclei of small size, with the chromatic areas tightly compact; and group 3 had two micronuclei of large size, the chromatic areas appearing loosely compacted or spongy. Later, special status was given to the group 2 syngens, and they became syngens 1, 2, and 3 of *P. multimicronucleatum*; and the single group 3 syngen became *P. jenningsi* (22). Autogamy, which occurs in all species of the *aurelia* complex, in *P. jenningsi*, and in most of the *P. multimicronucleatum* syngens, does not occur in syngen 2 of *P. multimicronucleatum* (21).

In the *aurelia* complex, five different esterases can be distinguished on the basis of their substrate specificity, sensitivity to the inhibitor eserine sulfate, and their response to different conditions of growth (type of medium, presence of acetate, presence of bacteria, etc.) (7, 10, 13). These five esterases are: Esterase A (EstA), Esterase B (EstB), Cathodal Esterase C ($EstC_C$), Anodal Esterase C ($EstC_A$), and Esterase D (EstD). Each type of esterase was found to vary independently, but at a low frequency, with both genetic as well as environmental factors determining the electrophoretic mobility or activity of individual esterases (8–10, 13, 18). For each type of esterase the electrophoretic form observed in the majority of stocks is referred to as the common subtype. The same subtype may be common to more than one species, since there are a limited number of subtypes found for each type of esterase: that is, six for EstA, 10 for EstB, and six for $EstC_C$ (4).

The acid phosphatases (Acp) are membrane bound in the *P. aurelia* species but can be resolved by special procedures (14). The overall Acp pattern was found to be somewhat similar for all species; however, differences in intensity and in mobility permitted the grouping of the species into eight subtypes (4).

Using *P. jenningsi*, the five syngens of *P. multimicronucleatum*, and one stock of *P. caudatum*, grown axenically, this paper addresses the following questions: Are the enzymes described above present in these species of *Paramecium*? If they are present, do these species have distinctive enzyme phenotypes? How much overlap (sharing of enzyme subtypes) is there between the syngens of *P. multimicronucleatum*? How much overlap is there between species in different species complexes (i.e., between species in the *P. aurelia*, *P. multimicronucleatum*, *P. jenningsi*, and *P. caudatum* species complexes)? Answers to these questions are important for assessing genetic distances within a second species complex and between species in different species complexes in *Paramecium* that all have similar body shape. Intraspecies variability, and the use of enzyme electrophoresis in assigning unidentified paramecia to *P. jenningsi* and *P. multimicronucleatum*, will be the focus of the last communication in this series (15).

MATERIALS AND METHODS

Stocks. Cultures of stocks of *P. jenningsi* (eight) and of *P. multimicronucleatum*, syngen 1 (five), syngen 2 (eighteen), syngen 3 (three), syngen 4 (two), and syngen 5 (one) were kindly sent from Indiana University by Myrtle V. Schneller. The cultures were growing on living bacteria and attempts were made to transfer all of them to axenic culture by methods described elsewhere (7, 12). We were successful in establishing axenic cultures from five stocks of *P. jenningsi*, and in *P. multimicronucleatum* from one stock only in syngen 1, 13 stocks in syngen 2, all three stocks in syngen 3, both stocks in syngen 4,

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and the one existing stock in syngen 5. A fourth stock in syngen 3 of *P. multimicronucleatum* (8MO) was collected by John Vandermeer (University of Michigan) in Costa Rica and was identified as belonging to this syngen by Sonneborn and Dippell (personal communication). It was easily transferred to axenic culture and has been described previously (1, 6). An axenic culture of stock pw_2 in *P. caudatum*, unknown as to syngen, was kindly sent by Raymond L. Napolitano. Axenic cultures of the stocks in *P. primaurelia*, *P. biaurelia*, and *P. tetraurelia* have been described previously (10, 14). Table I lists the stocks used in this paper, along with their geographical origin. Additional stocks in *P. jenningsi*, and in syngens 2, 3, and 4 of *P. multimicronucleatum*, will be described in a subsequent paper (15). During this study, axenic stocks were maintained in Maintenance Medium, which contains the same components as Axenic Medium with the addition of autoclaved bacteria. Details of the culture media are described in Allen & Nerad (12).

Growth of the cultures for extracts. Stocks were grown on Axenic Medium to the stationary phase (7–12 days, depending on the growth rate of the particular stock) in 3-liter flasks or 1-liter diphtheria toxin bottles at 23°C (13). Some of the stocks were also tested after growth in Maintenance Medium or Adaptation Medium (12).

Identification of esterases. Extracts of cell concentrates, harvested by centrifugation, were made by freeze-thawing. Electrophoresis of whole cell extracts was carried out in 12% starch gels using Electrostarch as previously described (5–7, 9). Two substrates were used: α-naphthyl propionate and α-naphthyl butyrate. Details of the histochemical procedures may be found in Allen & Gibson (7). Use of the inhibitor eserine sulfate has been described elsewhere (10).

Identification of acid phosphatases. Extracts of cell concentrates harvested by centrifugation were frozen-thawed before extraction with the detergents Triton X-100 and sodium deoxycholate (14). Electrophoresis was carried out in 12% starch gels using Electrostarch and a Tris-boric acid buffer, pH 7.7, containing sodium deoxycholate, to which Triton X-100 and a few drops of antifoam were added after cooking and before degassing (14). Conditions for running the gels and cutting and staining them after electrophoresis were the same as previously described (11, 14). The substrate used was sodium α-naphthyl acid phosphate.

RESULTS

General aspects. Each stock was grown under conditions that would optimize the visualization of the enzymes in the gels (see Materials and Methods). The mobilities of the esterases and acid phosphatases were compared after electrophoresis and staining of the gels. Extracts of each stock were run against extracts of all other stocks within a species or syngen, and then against extracts of one, or more, stocks from other species or syngens; and the mobilities of the enzymes were compared. Thirty stocks were tested, including those listed in Table I. An additional 18 stocks, unidentified as to species, were assigned to a particular species on the basis of their enzyme phenotypes. Except for syngen 2 in *P. multimicronucleatum* (where 26 stocks were screened) and 8MO in syngen 3, variants were a rarity. The intrasyngen and intraspecies comparisons will be taken up in a separate communication (15).

Esterases. The same five esterases identified in the *aurelia* complex were also found in most of the other species of *Paramecium* examined here. The A esterases react specifically with the substrate α-naphthyl propionate and are inhibited with 10^{-3} to 10^{-4} M eserine sulfate. EstA was found in all stocks of all species. As seen in the *P. aurelia* complex, isozymes were also observed in these species—at least two, and usually three isozymes. Enhancement of the more anodal isozymes by growth in Adaptation Medium, or in living bacteria, also occurred. The B esterases react more strongly with α-naphthyl butyrate than with α-naphthyl propionate and are inhibited by eserine sulfate when the concentration is raised to 10^{-2} M. EstB was found in all species, with the possible exception of syngen 3 of *P. multimicronucleatum*. Stock 8MO has a B esterase that migrates to a position similar to the slowest migrating A esterase (see Fig. 6 in Ref. 6), but the other syngen 3 stocks did not appear to have this esterase. In all other species and syngens, except for *P. caudatum*, at least two isozymes were present. Only one form was found in *P. caudatum*. The cathodally migrating C esterases have a slight preference for α-naphthyl propionate as substrate over α-naphthyl butyrate and are resistant to 10^{-2} M eserine sulfate. They are also sensitive to low concentrations of acetate in the growth medium; thus, an increase in enzyme activity is observed as the acetate component in Axenic Medium is used up (13). $EstC_C$ was found in all species, with the possible exception of *P. caudatum*. The anodally migrating C esterases have a substrate and inhibitor profile similar to that of the cathodal C esterases but are not sensitive to acetate in the growth medium. In the *aurelia* complex only some of the species have this type of esterase. Of the species of *Paramecium* examined here, only *P. jenningsi* had $EstC_A$, with all stocks showing it. The D esterases have a preference for α-naphthyl butyrate as substrate over α-naphthyl propionate and are resistant to 10^{-2} M eserine sulfate. They are sensitive to the component TEM-4T (tartaric acid esters of beef tallow monoglycerides) and to high concentrations of acetate in the growth medium. EstD was found in all the species examined here.

Comparisons of the mobilities of the esterases in the five syngens of *P. multimicronucleatum* are shown photographically (Fig. 1) and diagrammed (Fig. 2). Syngens 1 and 5 have identical phenotypes for all four esterases and have an esterase D similar in mobility to that found in syngen 4. Distinctive esterase phenotypes are found in syngens 2 and 3 and for three of the esterases in syngen 4.

The mobilities of the esterases in the five syngens of *P. multimicronucleatum* are compared with those observed in *P. jenningsi*, *P. caudatum*, *P. primaurelia*, *P. biaurelia*, and *P. tetraurelia* diagrammatically in Fig. 2. Photographs of some of the comparisons are shown in Fig. 3. In general, the mobilities of the A, B, and D esterases in the syngens of *P. multimicronucleatum* and in *P. caudatum* are greater than those observed for species of the *aurelia* complex, except for *P. tetraurelia* (and for the A esterases of *P. octaurelia*). More overlap with the *P.*

TABLE I. *List of stocks of* Paramecium *species used in present study.*

Species	Stock	Geographical origin
P. primaurelia	26	Stanford, CA
	90	Bethayres, PA
P. biaurelia	206	Oslo, Norway
P. tetraurelia	29	Hebbville, MD
P. caudatum	pw_2	?
P. jenningsi	4	Bangalore, India
	6	Avon Park, FL
P. multimicronucleatum		
Syngen 1	6	Apalachicola R., FL
Syngen 2	46	Lake Bogenham, MS
	49	Empire Range, Panama
Syngen 3	58	Bangkok, Thailand
	8MO	Turrialba, Costa Rica
Syngen 4	35	St. Johns Co., FL
Syngen 5	40	Tamiami Trail, FL

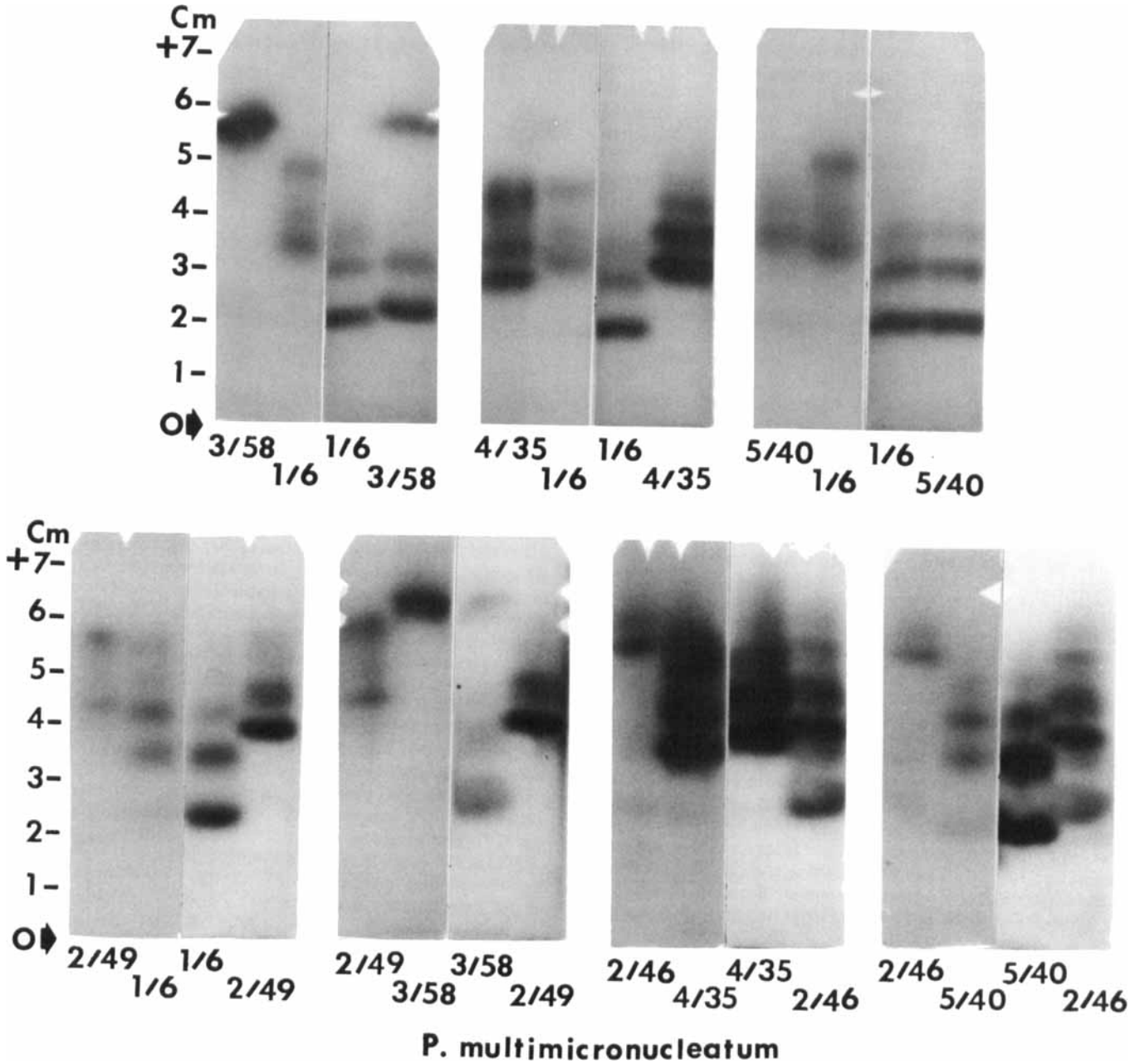

Fig. 1. Comparison of the A, B, C, and D esterases among the syngens of *Paramecium multimicronucleatum*. Right and left gels are mirror images. *Left gel:* α-naphthyl butyrate; *right gel:* α-naphthyl propionate. Only the anodal portion of the gels is shown. Syngens and stocks are designated by number/number (e.g., 1/6 refers to syngen 1, stock 6; 3/58 refers to syngen 3, stock 58; etc.). Distances of migration are marked off in centimeters from the origin (O) on the margin(s) of this and subsequent figures.

aurelia complex is observed for the mobilities of the A, B, C_A, and D esterases in *P. jenningsi* and in stock 8MO in syngen 3 of *P. multimicronucleatum*. When the cathodal C esterases are compared, a much greater spread in mobility is observed for the syngens of *P. multimicronucleatum*, *P. jenningsi*, and *P. caudatum* than for species in the *P. aurelia* complex—from slowly migrating forms in syngens 1, 3 and 5 in *P. multimicronucleatum* to those in *P. jenningsi* and syngen 4 of *P. multimicronucleatum* with mobilities similar to the $EstC_C$ in *P. sexaurelia*. A detailed comparison of the number of isozymes and their mobilities is given for EstA, EstB, $EstC_C$, and EstD in the different species of *Paramecium* in Table II. Where similarity to a species in the *P. aurelia* complex is noted, the most common subtype in that species is the one to which we are referring.

Acid phosphatases. The acid phosphatases in *Paramecium* appear to be membrane-bound since treatment with detergents is necessary to release them and special conditions must be used to resolve them during electrophoresis (4). This is true for all the species of *Paramecium* examined, although a couple of faint bands were seen in gels after simple freeze-thawing of extracts of *P. multimicronucleatum*, syngen 3.

Up to five zones of activity appear in the gels after electrophoresis of detergent-treated extracts from 13 of the 14 species

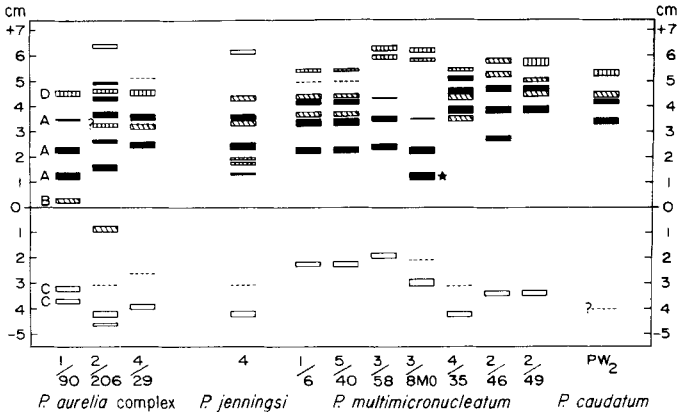

Fig. 2. Diagrams of the esterases in stocks of *P. primaurelia, P. biaurelia, P. tetraurelia, P. jenningsi, P. multimicronucleatum,* syngens 1–5, and *P. caudatum.* Species (syngens) and stocks are designated by number/number (e.g., 1/90 from the *P. aurelia* complex refers to *P. primaurelia,* stock 90; 1/6 from the *P. multimicronucleatum* complex refers to syngen 1, stock 6; etc.). Types of esterases are represented by the following symbols: ▬ = type A, ▭ = type B, ▭ = type C, ▭ = type D. The dashed lines indicate esterases of very low activity. The star indicates the position of the B esterase in Pm3/8MO (hidden from view by an A esterase isozyme).

examined in the *P. aurelia* complex (4). The pattern of bands has been treated as a unit because of the limited mobility and poor resolution of the enzyme forms in *Paramecium.* In the *P. aurelia* species the overall pattern was somewhat similar, although differences in intensity and mobility of individual zones were noted. Eight groups of species could be distinguished.

Using *P. primaurelia* as a "standard," comparisons were made of the Acp patterns found in each of the syngens of *P. multimicronucleatum, P. jenningsi,* and *P. caudatum* (Fig. 4). The zones of acid phosphatase activity in *P. primaurelia* are labeled A, B, C, D, and E in order of increasing mobility in Fig. 5 where the patterns of activity are diagrammed. The Acp patterns of syngens 1, 2, 4, and 5 of *P. multimicronucleatum* and of *P. jenningsi* and in syngens 2, 3, and 4 of *P. multimicronucleatum aurelia* complex in general, although the differences in individual zones are somewhat greater than those seen for species within that complex. None of the species examined here had a similar pattern, except for *P. multimicronucleatum,* syngens 2 and 4. Note that syngens 1 and 5 have different Acp phenotypes. Particularly interesting are the distinctive, but different, patterns of acid phosphatase activity seen for *P. caudatum* and for syngen 3 of *P. multimicronucleatum.* Moreover, all four stocks in syngen 3, including 8MO, had the same distinctive pattern. This pattern is so distinctive it should be an excellent diagnostic tool

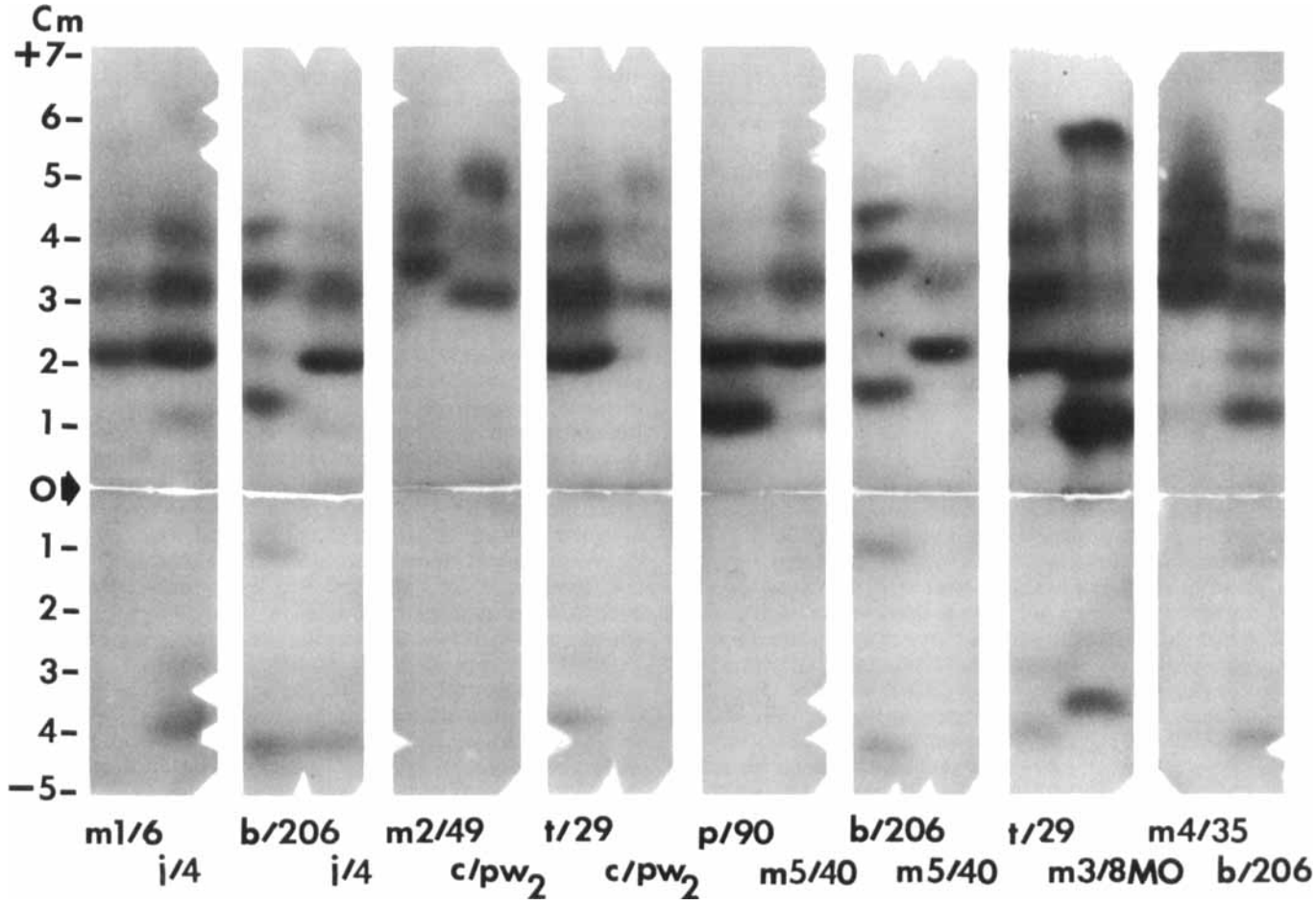

Fig. 3. Comparison of the A, B, C, and D esterases in *P. jenningsi* (j), *P. caudatum* (c), *P. multimicronucleatum,* syngens 1, 2, 3, 4, and 5 (m1, m2, m3, m4, m5), and *P. primaurelia* (p), *P. biaurelia* (b), and *P. tetraurelia* (t). Stocks are designated by the number following the species code letter (e.g., j/4 refers to *P. jenningsi,* stock 4; m3/8MO refers to *P. multimicronucleatum,* syngen 3, stock 8MO; etc.). $EstC_C$ is extremely faint in p/90, m1/6, m2/49, m4/35, and m5/40, and may not be present in c/pw_2. Substrate: α-naphthyl propionate.

TABLE II. *Comparison of the number of isozymes and their mobilities for four enzymes in different species of* Paramecium.

Species	Stock	Esterase A		Esterase B	
		Number	Mobility	Number	Mobility
P. primaurelia	90	3	Similar to *P. triaurelia, P. pentaurelia, P. septaurelia, P. undecaurelia, P. tredecaurelia, P. quadecaurelia*	1	Similar to *P. triaurelia, P. pentaurelia*
P. biaurelia	206	5–6	Unique (hypervariable in *P. biaurelia*)	1	Unique
P. tetraurelia	29	2	Similar to *P. octaurelia*	1	Unique
P. jenningsi	4	3	A-2~A-1, A-3~A-2 in *P. tetraurelia*	2	Unique
P. multimicronucleatum-1	6	3	Similar to *P. multimicronucleatum*, 5/40	2	Similar to *P. multimicronucleatum*, 5/40
P. multimicronucleatum-5	40	3	Similar to *P. multimicronucleatum*, 1/6	2	Similar to *P. multimicronucleatum*, 1/6
P. multimicronucleatum-3	58	3	Similar to *P. tetraurelia* (A-1~A-1, A-2~A-2)	?	Not present?
P. multimicronucleatum-3	8MO	3	Similar to *P. primaurelia, P. triaurelia, P. pentaurelia, P. septaurelia, P. undecaurelia, P. tredecaurelia, P. quadecaurelia*	1	Unique
P. multimicronucleatum-4	35	3	Unique (A-1 possibly~to A-1 in *P. multimicronucleatum*, 2/49; to A-2 in *P. multimicronucleatum*, 2/46)	2	Unique
P. multimicronucleatum-2	46	3	A-2~A-1, A-3~A-2 in *P. multimicronucleatum*, 2/49	2	Unique
P. multimicronucleatum-2	49	2	A-1~A-2, A-2~A-3 in *P. multimicronucleatum*, 2/46	2	Unique
P. caudatum	pw_2	2	A-1~A-2, A-2~A-3 in *P. multimicronucleatum*, 1/6, 5/40	1	Similar to B-2 in *P. multimicronucleatum*, 1/6, 5/40

for assigning new stocks to syngen 3. The distinctive pattern for stock pw_2 of *P. caudatum* may be diagnostic of whichever syngen it belongs to in that species complex.

DISCUSSION

Common presence of certain enzymes in species of Paramecium. The five esterases and the acid phosphatases first characterized in species of the *P. aurelia* complex are also found in *P. jenningsi*, and three to four of the esterases and the acid phosphatases are found in the *P. multimicronucleatum* species complex and in *P. caudatum*. Thus, these enzymes appear to be widely distributed in the genus *Paramecium*, although these particular species complexes are morphologically related in that they all have a "cigar-shaped" body (20). It would be interesting to see if they are also present in morphologically less related species complexes, such as *P. bursaria*.

The *Tetrahymena pyriformis* species complex also has esterases and acid phosphatases, but their characteristics set them apart from the enzymes seen in *Paramecium* (3, 11, 16). The esterases are a family of enzymes that differ in substrate specificity and sensitivity to various inhibitors and activators. Structural genes have been identified for two sets of esterase isozymes. The esterase-1 isozymes are propionylesterases, activated by sodium taurocholate, sodium cholate, and Triton X-100, and inhibited by eserine sulfate (10^{-4} M), L-cysteine, and mercaptoethanol. Each homozygote has five or six "secondary" isozymes (conformers) that segregate as a unit in crosses, but are sensitive to growth conditions. The esterase-2 isozymes are butyrylesterases, insensitive to eserine sulfate or sodium cholate, but inactivated by p-chloromercurobenzoic acid, and are insensitive to growth conditions. The acid phosphatases are also a family of enzymes, with different gene products being displayed. After simple freeze-thawing, some 18–20 well resolved bands appear with a much greater spread in their mobility than the detergent-released forms seen in *Paramecium* gels.

Distinctiveness of constellation of enzyme phenotypes. The species in the *aurelia* complex can be grouped by mobility into specific subtypes for each enzyme. There are six subtypes for EstA, 10 subtypes for EstB, six subtypes for $EstC_C$, and eight subtypes for Acp (4). New subtypes are observed in the species examined here—with five additional subtypes seen for EstA, six additional subtypes for EstB, three additional subtypes for $EstC_C$, and six additional subtypes for Acp. In general, the new subtypes for EstA and EstB have greater mobility than those observed in the *aurelia* complex, while two of the three new subtypes for $EstC_C$ have a slower mobility than any of those found in the *aurelia* complex. Two of the new Acp subtypes, present in syngen 3 of *P. multimicronucleatum* and in *P. caudatum*, differ radically in mobility and pattern from the others.

General uniqueness of enzyme phenotypes among syngens of P. multimicronucleatum. In *P. multimicronucleatum*, the enzyme phenotypes seen in the five syngens show a wide range in

TABLE II. *Continued.*

Species	Stock	Esterase C_C		Esterase D	
		Number	Mobility	Number	Mobility
P. primaurelia	90	2	Similar to *P. triaurelia, P. pentaurelia, P. novaurelia, P. decaurelia, P. dodecaurelia*	1	Similar to *P. biaurelia, P. tetraurelia, P. pentaurelia, P. sexaurelia, P. octaurelia* (possibly others from *aurelia* complex)
P. biaurelia	206	2	Similar to *P. octaurelia*	2?	One similar to *P. primaurelia, P. tetraurelia, P. pentaurelia, P. sexaurelia, P. octaurelia* (possibly others from *aurelia* complex)
P. tetraurelia	29	1	Unique	1	Similar to *P. primaurelia, P. biaurelia, P. pentaurelia, P. sexaurelia, P. octaurelia* (possibly others from *aurelia* complex)
P. jenningsi	4	1	Similar to C-1 in *P. biaurelia* (*P. sexaurelia*); to *P. multimicronucleatum*-4	1	Unique
P. multimicronucleatum-1	6	1	Similar to *P. multimicronucleatum*, 5/40	1	Similar to *P. multimicronucleatum*, 5/40, 4/35
P. multimicronucleatum-5	40	1	Similar to *P. multimicronucleatum*, 1/6	1	Similar to *P. multimicronucleatum*, 1/6, 4/35
P. multimicronucleatum-3	58	1	Unique	2	Unique
P. multimicronucleatum-3	8MO	1	Similar to *P. septaurelia, P. undecaurelia, P. tredecaurelia*	2	Slightly slower than doublet in *P. multimicronucleatum*, 3/58
P. multimicronucleatum-4	35	1	Similar to C-1 in *P. biaurelia* (*P. sexaurelia*); to *P. jenningsi*	1	Similar to *P. multimicronucleatum*, 1/6, 5/40
P. multimicronucleatum-2	46	1	Unique (similar to *P. multimicronucleatum*, 2/49)	?	Not present?
P. multimicronucleatum-2	49	1	Unique (similar to *P. multimicronucleatum*, 2/46)	1	Unique
P. caudatum	pw_2	1?	Not present?	1	Unique? (possibly similar to *P. multimicronucleatum*, 1/6, 4/35, 5/40)

similarity—from zero similarity for syngen 3 and the other syngens to 80% similarity (4/5 enzymes) for syngens 1 and 5. For the most part the similarity between syngens is limited, with syngen 4 sharing a D esterase with syngens 1 and 5 and Acp with syngen 2. Zero similarity is also observed for syngen 2 and syngens 1 and 5. A range of similarity coefficients was observed for the *aurelia* complex—from 81% for *P. primaurelia* and *P. pentaurelia* to 9–10% for eight of the comparisons (4). None of the paired comparisons showed zero similarity; however, this observation was made for some of the species in the *T. pyriformis* complex (17). Separate species status was given to the syngens in *P. aurelia* and in *T. pyriformis* when the magnitude of the molecular distances was realized, enzyme electrophoresis could be used to identify stocks, and stocks could be frozen and stored in liquid nitrogen (19, 23). By the same reasoning, the syngens of *P. multimicronucleatum* should also be considered as separate species (although they are not formally named in the present paper).

Limited sharing of enzyme phenotypes between species complexes. There is limited sharing of enzyme phenotypes in species of the *P. multimicronucleatum* complex with species in the *aurelia* complex. Syngen 4 appears to have an $EstC_C$ similar in mobility to one found in *P. sexaurelia*, while syngen 3, stocks 12, 57, and 58, has EstA isozymes similar in mobility to those seen in *P. tetraurelia* and *P. octaurelia*. Stock 8MO shows greater similarity, with an EstA common to a group of species in *P. aurelia* that includes *P. primaurelia, P. triaurelia, P. pentaurelia, P. septaurelia, P. undecaurelia, P. tredecaurelia,* and *P. quadecaurelia* and an $EstC_C$ common to a group of species in *P. aurelia* that includes *P. septaurelia, P. undecaurelia,* and *P. tredecaurelia.*

P. jenningsi has an unusual set of EstA isozymes. Typically, the slowest isozyme is the most active, with the more rapidly migrating isozymes falling off in activity. In *P. jenningsi,* the slowest EstA isozyme is the *least* active. Moreover, the second and third isozymes have activities and mobilities similar to those observed in *P. tetraurelia.* The cathodal C esterase is similar in mobility to the ones observed in syngen 4 of *P. multimicronucleatum* and *P. sexaurelia.* Thus, there is limited sharing of enzyme phenotypes of *P. jenningsi* with both the *P. aurelia* and *P. multimicronucleatum* species complexes.

The one stock examined in *P. caudatum* shows no similarity to any of the species in the *P. aurelia* complex in its enzyme phenotypes. It appears to lack $EstC_C$. It does have an EstD, which is similar in mobility to that seen in species 1, 4, and 5 in *P. multimicronucleatum.* This differentiation from other *Paramecium* species complexes also appears to be true for the lactate dehydrogenase isozymes (2). The electrophoretic pattern, similar in four syngens of *P. caudatum,* was distinct from species in other species complexes of *Paramecium.*

Although the data include comparison of only six enzymes, it appears that the degree of similarity between species in the

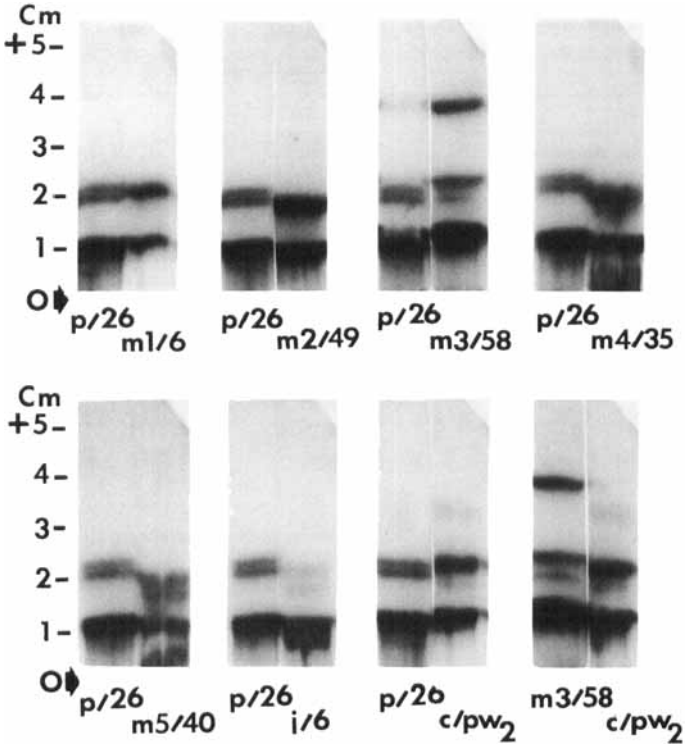

Fig. 4. Comparison of the acid phosphatases in *P. jenningsi, P. caudatum, P. multimicronucleatum,* syngens 1–5, and *P. primaurelia.* See legend to Fig. 3 for code designation of stocks.

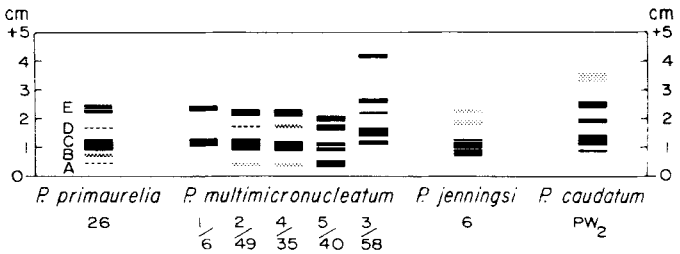

Fig. 5. Diagrams of the acid phosphatases in stocks of *P. primaurelia, P. multimicronucleatum,* syngens 1–5, *P. jenningsi,* and *P. caudatum.* Syngens and stocks in *P. multimicronucleatum* are designated by number/number (e.g., 1/6 refers to syngen 1, stock 6; etc.). The relative intensities of the bands are indicated by the intensity of shading. Dashed lines indicate bands that are extremely faint and are not always seen on the gels.

P. jenningsi, P. multimicronucleatum, P. caudatum, and *aurelia* complexes is low. With one or two exceptions, very few subtypes are held in common. This suggests that the molecular distances between species complexes may be greater than between species within a complex. However, more enzymes need to be examined before any solid conclusions can be reached.

The next paper will compare intraspecies variability in *P. jenningsi* and in syngens 2, 3, and 4 of *P. multimicronucleatum* with what is known for species in the *aurelia* complex (15). We will also show how enzyme electrophoresis can be used to assign unidentified paramecia to a particular species.

LITERATURE CITED

1. Adams, J. & Allen, S. L. 1975. Genetic polymorphism and differentiation in *Paramecium, in* Markert, C. L., ed., *Isozymes IV. Genetics and Evolution,* Academic Press, New York, pp. 867–882.

2. Agatsuma, T. & Tsukii, Y. 1980. Genetic control of lactate dehydrogenase isozymes in *Paramecium caudatum. Biochem. Genet.,* **18**: 77–85.

3. Allen, S. L. 1968. Genetic and epigenetic control of several isozymic systems in *Tetrahymena. Ann. N.Y. Acad. Sci.,* **151**: 190–207.

4. Allen, S. L., Adams, J. & Rushford, C. L. 1983. Interspecies relationships in the *Paramecium aurelia* complex: acid phosphatase variation. *J. Protozool.,* **30**: 143–147.

5. Allen, S. L., Byrne, B. C. & Cronkite, D. L. 1971. Intersyngenic variations in the esterases of bacterized *Paramecium aurelia. Biochem. Genet.,* **5**: 135–150.

6. Allen, S. L., Farrow, S. W. & Golembiewski, P. A. 1973. Esterase variations between the 14 syngens of *Paramecium aurelia* under axenic growth. *Genetics,* **73**: 561–573.

7. Allen, S. L. & Gibson, I. 1971. Intersyngenic variations in the esterases of axenic stocks of *Paramecium aurelia. Biochem. Genet.,* **5**: 161–181.

8. ——— 1975. Syngenic variations for enzymes of *Paramecium aurelia, in* Markert, C. L., ed., *Isozymes IV. Genetics and Evolution,* Academic Press, New York, pp. 883–899.

9. Allen, S. L. & Golembiewski, P. A. 1972. Inheritance of esterases A and B in syngen 2 of *Paramecium aurelia. Genetics,* **71**: 469–475.

10. Allen, S. L., Lau, E. T., Nerad, T. A. & Rushford, C. L. 1982. Esterase variants in four species of the *Paramecium aurelia* complex. *J. Protozool.,* **29**: 604–611.

11. Allen, S. L., Misch, M. S. & Morrison, B. M. 1963. Variations in the electrophoretically separated acid phosphatases of Tetrahymena. *J. Histochem. Cytochem.,* **11**: 706–719.

12. Allen, S. L. & Nerad, T. A. 1978a. Method for the simultaneous establishment of many axenic cultures of *Paramecium. J. Protozool.,* **25**: 134–139.

13. ——— 1978b. Effect of acetate on esterase C activity during the growth cycle of *Paramecium. J. Protozool.,* **25**: 273–279.

14. Allen, S. L., Nerad, T. A. & Rushford, C. L. 1983. Intraspecies variability in the esterases and acid phosphatases of four species of the *Paramecium aurelia* complex. *J. Protozool.,* **30**: 131–143.

15. Allen, S. L., Rushford, C. L., Nerad, T. A. & Lau, E. T. 1983. Intraspecies variability in the esterases and acid phosphatases of *Paramecium jenningsi* and *Paramecium multimicronucleatum*: assignment of unidentified paramecia; comparison with the *P. aurelia* complex. *J. Protozool.,* **30**: 155–163.

16. Allen, S.L. & Weremiuk, S. L. 1971. Intersyngenic variations in the esterases and acid phosphatases of *Tetrahymena pyriformis. Biochem. Genet.,* **5**: 119–133.

17. Borden, D., Miller, E. T., Whitt, G. S. & Nanney, D. L. 1977. Electrophoretic analysis of evolutionary relationships in *Tetrahymena. Evolution,* **31**: 91–102.

18. Cavill, A. & Gibson, I. 1972. Genetic determination of esterases of syngens 1 and 8 in *Paramecium aurelia. Heredity,* **28**: 31–37.

19. Nanney, D. L. & McCoy, J. W. 1976. Characterization of the species of the *Tetrahymena pyriformis* complex. *Trans. Am. Microsc. Soc.,* **95**: 664–682.

20. Sonneborn, T. M. 1957a. Breeding systems, reproductive methods and species problems in protozoa, *in* Mayr, E., ed., *The Species Problem,* Am. Assoc. Adv. Sci. Symp., Washington, D.C., pp. 155–324.

21. ——— 1957b. Varieties 13, 15 and 16 of the *P. aurelia-multimicronucleatum* complex. (Abstr.) *J. Protozool.,* **4**(Suppl.): 21.

22. ——— 1958. Classification of syngens of the *Paramecium aurelia-multimicronucleatum* complex. (Abstr.) *J. Protozool.,* **5**(Suppl.): 17–18.

23. ——— 1975. The *Paramecium aurelia* complex of fourteen sibling species. *Trans. Am. Microsc. Soc.,* **94**: 155–178.

24. Sonneborn, T. M. & Dippell, R. V. 1957. The *Paramecium aurelia-multimicronucleatum* complex. (Abstr.) *J. Protozool.,* **4**(Suppl.): 21.

25. Tait, A. 1970. Enzyme variation between syngens in *Paramecium aurelia. Biochem. Genet.,* **4**: 461–470.

26. ——— 1978. Species identification in protozoa: glucosephosphate isomerase variation in the *Paramecium aurelia* group. *Biochem. Genet.,* **16**: 945–955.

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