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Intraspecies Variability in the Esterases and Acid Phosphatases of *Paramecium jenningsi* and *Paramecium multimicronucleatum*: Assignment of Unidentified Paramecia; Comparison with the *P. aurelia* Complex[1]

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ABSTRACT. Enzyme electrophoresis was exploited to identify stocks of paramecia previously not identified to particular species. Stocks collected in India and one from Panama belong to *Paramecium jenningsi*, while others collected in Panama or in Brazil are assignable to syngen 2 of *P. multimicronucleatum* on the basis of similarity of their esterase and acid phosphatase phenotypes. Inclusion of these doubled the numbers of stocks available in the two species, thereby facilitating examination of intraspecies variation and comparison of particular features of intraspecies variation found for the *P. aurelia* complex. Variant stocks were observed in *P. jenningsi* and in syngens 2, 3, and 4 of *P. multimicronucleatum*. In some cases the variant lacked the enzyme; in others, a change in mobility of the enzyme occurred that resulted in an electrophoretic form similar to one common in another species. Unique phenotypes were displayed by the variants of syngen 2 in *P. multimicronucleatum*. Hypervariability for Esterase B was observed in this syngen, where, in addition, several subtypes were seen for three other esterases. Unique phenotypes and hypervariability were also noted in *P. biaurelia*. Clustered variations were observed in these species and in the *P. aurelia* species. Unlike the situation for members of the *aurelia* complex, where lack of geographical differentiation between stocks in the same species is a unique feature, some such differentiation does occur in *P. multimicronucleatum*-2. The frequency of variant stocks in *P. jenningsi* was similar to that observed in the *aurelia* sibling species. In contrast, a significantly higher frequency of variant stocks was found in syngens 2, 3, and 4 of *P. multimicronucleatum*.

ENZYME electrophoresis has been exploited to identify stocks belonging to different species in the *Paramecium aurelia* complex (4, 12, 22, 23). Provided the frequency of enzyme phenotypes shared by different species is low, assignment of stocks to species can be carried out unambiguously (2, 23). Separate species status was given to the syngens of (the old) *P. aurelia* when it became possible to identify stocks by enzyme electrophoresis and they could be frozen and stored in liquid nitrogen (19). Each species has a distinctive array of enzyme phenotypes. Comparison of enzyme phenotypes between species has shown that the molecular distances between species are large despite their lack of morphological differentiation (2).

A number of stocks collected in Panama, Brazil, and India in 1957 to the mid-1960's were suspected of belonging to either *Paramecium jenningsi* or *P. multimicronucleatum*, but for reasons no longer possible to determine, they had never been assigned to species (Sonneborn and Schneller, personal communication). These stocks were sent to us so we could identify them using the enzyme electrophoresis techniques. This has been accomplished, and we will show that some of these collected stocks belong to *P. jenningsi* and others to syngen 2 of *P. multimicronucleatum*.

Five different esterases in members of the *aurelia* complex can be distinguished on the basis of their substrate specificity, sensitivity to the inhibitor eserine sulfate, and their response to different conditions of growth (5, 8, 11). All, or most, of these five esterases are also present in species of the *P. jenningsi, P. multimicronucleatum,* and *P. caudatum* species complexes (13): 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, with genetic as well as environmental factors determining the electrophoretic mobility or activity of individual esterases (6–8, 11, 13, 14). For eash esterase, each species has a commonly observed subtype (of a particular mobility) that may, or may not, be shared with other species. Each species (or syngen) has a distinctive constellation of esterase subtypes. Except for syngents 1 and 5—of *P. multimicronucleatum*—there was little or no similarity in esterase phenotypes among syngens of that species (13). Moreover, only limited sharing of subtypes was observed among species in the *P. aurelia, P. jenningsi, P. multimicronucleatum,* and *P. caudatum* species complexes (13).

The acid phosphatases (Acp) are membrane-bound in the *P. aurelia* complex, the *P. multimicronucleatum* complex, *P. jenningsi,* and *P. caudatum,* but can be resolved by special procedures (2, 12, 13). The overall pattern was found to be somewhat similar in most of the species of the *aurelia* complex, four of the syngens in *P. multimicronucleatum,* and *P. jenningsi*; but differences within this pattern permitted eight groupings of the 14 species in the *aurelia* complex and differentiation of syngens 1 from 5 and from 2, 4 in *P. multimicronucleatum* and from *P. jenningsi.* Distinctive new patterns were observed in syngen 3 of *P. multimicronucleatum* and in *P. caudatum.*

When intraspecies variation for the esterases and acid phosphatases was examined in a large number of stocks in four species of the *aurelia* complex, we found that the majority of the esterase variants in three of the species (*P. primaurelia, P. tetraurelia, P. octaurelia*) had an electrophoretic mobility similar to a common subtype or a variant in another species, while in *P. biaurelia* the mobilities of most of the variants were unique (8). In all four species, the acid phosphatase variants appeared to have unique phenotypes (12). In spite of the cosmopolitan distribution of these species, a special feature noted was the lack of geographical differentiation of stocks showing both common as well as variant enzyme phenotypes. We also observed clustering of variations in some of the variant stocks. The frequency of variation was low but similar, in *P. primaurelia, P. tetraurelia,* and *P. octaurelia,* but higher in *P. biaurelia* (12).

The present study is concerned with intraspecies variability in *P. jenningsi* and syngens 2, 3, and 4 of *P. multimicronucleatum* and was undertaken to see which, if any, of the features observed for the *aurelia* complex also apply to other species complexes in the genus *Paramecium.* The paper addresses the following specific questions: Do the variants in *P. jenningsi* and *P. multimicronucleatum* have unique phenotypes, or, are the changes in mobility similar to subtypes found in other species? Is there clustering of variations? Is there geographical differentiation of stocks showing different subtypes within a species? What is the frequency of intraspecies variation? The assignment

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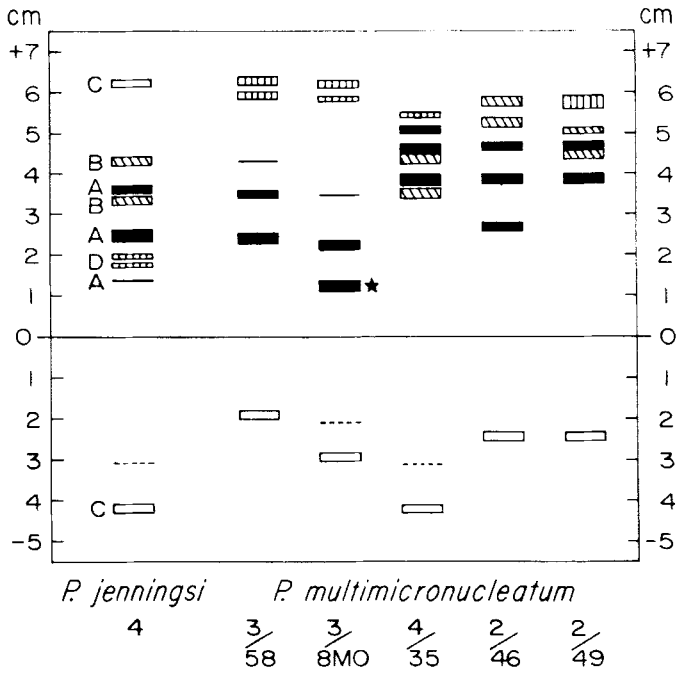

Fig. 1. Diagrams of the esterases in stocks of *Paramecium jenningsi* and of *P. multimicronucleatum*, syngens 3, 4, and 2. Types of esterases are represented by the following symbols: ▬ = type A, ▭ = type B, ▭ = type C, ▭ = type D. The dashed lines indicate esterases of very low activity. The star indicates the position of the B esterase in Pm 3/8MO (hidden from view by an A esterase isozyme). Distances in migration are marked off in centimeters from the origin (O) on the margin(s) of this and subsequent figures.

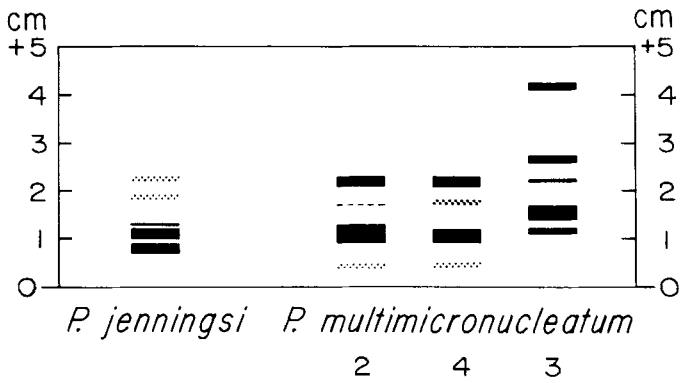

Fig. 2. Diagrams of the acid phosphatases in stocks of *P. jenningsi* and *P. multimicronucleatum*, syngens 2, 4, and 3. The relative intensities of the bands are indicated by the intensity of shading. Dashed lines indicate bands that are extremely faint and are not always seen on the gels.

of additional stocks to *P. jenningsi* and *P. multimicronucleatum*-2 was important in facilitating this study since only a few stocks in these species were available in the Sonneborn Collection.

MATERIALS AND METHODS

Stocks. Cultures of 37 stocks of *P. jenningsi* and of syngens 1–5 of *P. multimicronucleatum*, growing on living bacteria, were kindly sent from Indiana University by Myrtle V. Schneller. We were successful in establishing axenic cultures from five stocks in *P. jenningsi*, and one stock in syngen 1, thirteen in syngen 2, three in syngen 3, two in syngen 4, and one in syngen 5 of *P. multimicronucleatum*. A fourth stock in syngen 3 of *P. multimicronucleatum* (8MO), collected by John Vandermeer (University of Michigan) in Costa Rica, 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, 4). We were also sent 20 stocks that had not been assigned to species but were suspected of belonging to *P. jenningsi* or *P. multimicronucleatum*. The P series included 12 stocks from five collections made by Dr. David Sonneborn in Panama in March 1957. The S series included four stocks collected by Dr. B. R. Seshachar in India, which were received in April 1957 at Indiana University. The B1A series included four stocks collected in Brazil and reaching Indiana in the early to mid-1960's. We were successful in transferring all stocks except one from Panama (P11-1) and one from Brazil (B1A4) to axenic culture by methods described elsewhere (5, 10). 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 (10).

Growth of the cultures for extracts. Stocks were grown in 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 (11). Some of the stocks were also tested after growth in Maintenance Medium or Adaptation Medium (10).

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 (3–5, 7). Two substrates were used: α-naphthyl propionate and α-naphthyl butyrate. Details of histochemical procedures and use of inhibitor eserine sulfate are described elsewhere (5, 8).

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 (12). 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 (12). Conditions for running the gels and cutting and staining them after electrophoresis were the same as previously described (9, 12). 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 for 44 stocks after electrophoresis of extracts and staining of the gels. The esterases of each stock were tested for substrate preference and for eserine inhibition to identify the five types of esterases. Extracts of stocks previously identified as to species and/or syngen were run against extracts of all other stocks in the same grouping, and the mobilities of the enzymes were compared. Extracts of unidentified stocks were run against each other and extracts of stocks known to belong to a particular species or syngen. After tentative assignment to a particular species or syngen, they were run against extracts of other stocks in the same group. The species assignment of these stocks was subsequently confirmed on the basis of morphological criteria (Daggett and Nerad, personal communication).

TABLE I. *Enzyme subtypes of stocks of* Paramecium jenningsi.[a]

Stock	EstA	EstB	$EstC_C$	$EstC_A$	EstD	Acp	Geographical origin
2	I	I	I	I	I	I	?
3	I	I	I	I	I	I	Barro Colorado Island, Panama
4	I	I	I	I	I	I	Bangalore, India
5	I	I	I	I	I	I	Bangalore, India
6	I	I	I	I	—	I	Avon Park, FL
P3-1	I	I	I	I	I	I	Panama
S11a-f	I	I	I	I	I	I	India
S11c	I	I	I	I	I	I	India
S15	I	I	I	I	I	I	India
S16	I	I	I	I	I	I	India

[a] Key to abbreviations: EstA = Esterase A, EstB = Esterase B, $EstC_C$ = Cathodal C esterase, $EstC_A$ = Anodal C esterase, EstD = Esterase D, Acp = Acid phosphatase.

The five esterases of *Paramecium* differ in their substrate specificity, sensitivity to the inhibitor eserine sulfate, and their response to particular growth conditions (13). The A esterases react specifically with the substrate α-naphthyl propionate, are inhibited with 10^{-3}–10^{-4} M eserine sulfate, and display isozymes, the more anodal forms being enhanced by growth in Adaptation Medium or in living bacteria. The B esterases react more strongly with α-naphthyl butyrate than with α-naphthyl propionate, are inhibited by eserine sulfate when the concentration is raised to 10^{-2} M, and two, or more, isozymes are present in *P. jenningsi* and *P. multimicronucleatum*. The cathodally migrating C esterases have a slight preference for α-naphthyl propionate as substrate over α-naphthyl butyrate, are resistant to 10^{-2} M eserine sulfate, and are 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. 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. The D esterases prefer α-naphthyl butyrate as substrate over α-naphthyl propionate, are resistant to 10^{-2} M eserine sulfate, and are sensitive to the component TEM-4T (tartaric acid esters of beef tallow monoglycerides) and to high concentrations of acetate in the growth medium. The mobilities of the esterases in *P. jenningsi* and syngens 2, 3, and 4 of *P. multimicronucleatum* are compared (Fig. 1).

The acid phosphatases in *Paramecium* are released from extracts by treatment with detergents and resolved by addition of detergents to the gels during electrophoresis (13). Up to five zones of activity may appear in the gels; however, the pattern of bands is treated as a unit because of the limited mobility and poor resolution of the enzyme forms in *Paramecium*. The mo-

TABLE II. *Enzyme subtypes of stocks of* Paramecium multimicronucleatum, *syngens 3 and 4*.[a,b]

Stock	EstA	EstB	$EstC_C$	EstD	Acp	Geographical origin
3/12	I	—	I	I	I	Cuernavaca, Mexico
57	I	—	I	I	I	Luquillo Exptl. Forest, Puerto Rico
58	I	—	I	I	I	Bangkok, Thailand
8MO	II	I	II	II	I	Turrialba, Costa Rica
4/35	I	I	I	I	I	St. Johns Co., FL
37	I	I	I	—	I	Mustang Creek, TX

[a] Key to abbreviations: see Table I.

[b] Subtypes in syngen 3 are not similar to any of the subtypes in syngen 4.

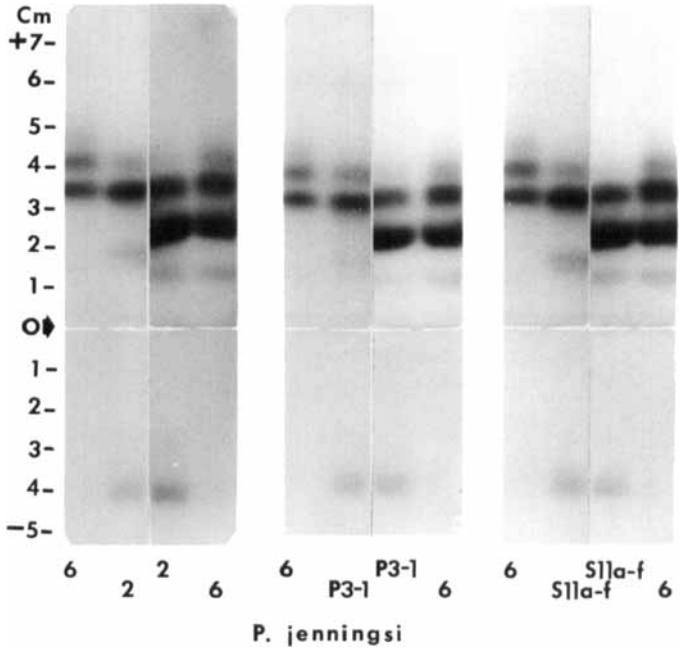

Fig. 3. Comparison of the A, B, C, and D esterases among stocks of *P. jenningsi*. Substrate for the gel on *left:* α-naphthyl butyrate; on *right:* α-naphthyl propionate. Note that right and left gels are mirror images in comparing stocks with the two substrates. See Fig. 1 for positions of the various types of esterases. $EstC_C$ is extremely faint in stock 6, but can be seen with longer incubations.

TABLE III. *Enzyme subtypes of stocks of* Paramecium multimicronucleatum, *syngen 2*.[a]

Stock	EstA	EstB	$EstC_C$	$EstC_A$	EstD	Acp	Geographical origin
4	I	II?	I	—	I	I	Joshua Creek, FL
9	III	III	I	—	III?	I	Seattle, WA
11	II	I	Ia	—	—	I	Wellesley, MA
17	I	I	I	—	—	I	Nottaway R., VA
18	I	I	I	—	—	I	Worchester Co., MD
20	I	II	I	—	—	I	Edisto R., SC
25	I	II?	I	—	—	nt[b]	Sussex Co., DE
42	I	I	I	—	—	I	St. Lucie Co., FL
43	II?	IV	Ib	—	II	I	Lake Ella, Tallahassee, FL
44	I	II	I	—	—	nt[b]	Mustang R., TX
46	II	I	I	—	—	I	Lake Bogenham, MS
48	I	III	I	—	III	I	Helmville, PA
49	I	V	I	—	III	I	Empire Range, Panama
P2-1	IV	V	I	—	III	I	Panama
P2-3	I	V	I	—	III	I	Panama
P7-1	V	V	II	—	III	I	Panama
P7-2	I	V	I	—	III	I	Panama
P11-2	I	V	I	—	III	I	Panama
P11-3	I	V	I	—	III	I	Panama
P11-4	I	V	I	I	III	I	Panama
P16-1	I	V	Ia	—	III	I	Panama
P16-2	I	V	Ia	—	III	I	Panama
P16-3	I	V	Ia	—	III	I	Panama
B1A-2	I	VI	Ia	—	III	I	Brazil
B1A-6	I	VI	Ia?	—	III	I	Brazil
B1A-7	I	VII	Ia	II	III	I	Brazil

[a] Key to abbreviations: see Table I.

[b] nt = not tested.

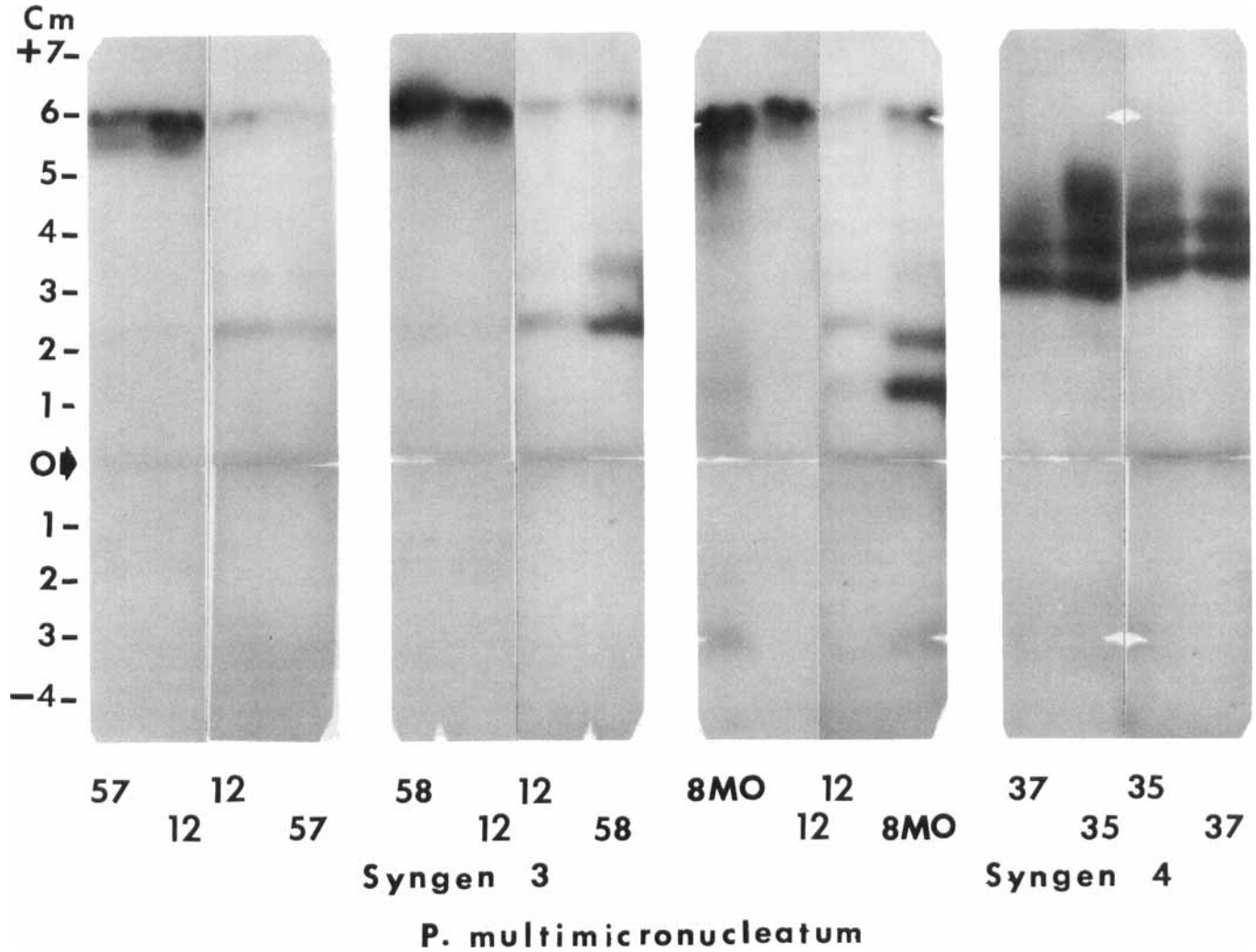

Fig. 4. Comparison of the A, B, C, and D esterases among stocks of *P. multimicronucleatum*, syngens 3 and 4. Substrate for the gel on *left:* α-naphthyl butyrate; on *right:* α-naphthyl propionate. The incubation time for the gels on the left was twice that for the gels on the right. See Fig. 1 for positions of the various types of esterases. $EstC_C$ is extremely faint in *P. multimicronucleatum*, except for certain stocks, such as 3/8MO.

bilities of the acid phosphatases in *P. jenningsi* and syngens 2, 3, and 4 of *P. multimicronucleatum* are compared (Fig. 2).

P. jenningsi. Stocks 2, 3, 4, 5, and 6 have EstA, EstB, $EstC_C$, $EstC_A$, and EstD esterases of identical mobility— with one exception. Stock 6 lacks the EstD esterases, a doublet that migrates about 2 cm toward the anode (see Fig. 3, left gel, for stocks 2 and 6). The acid phosphatases have a similar pattern in all five stocks.

All four of the unidentified stocks from the S series (S11a-f, S11c, S15, S16) and one of the unidentified stocks from the P series (P3-1) could be assigned to *P. jenningsi* on the basis of their enzyme phenotypes. The esterases of P3-1 and S11a-f are compared to stock 6 in Fig. 3, where it can be seen that they are identical except for the presence of the D esterases in P3-1 and the S series.

Table I shows that all 10 stocks have similar phenotypes for the six enzymes (except for stock 6's lacking EstD). This species appears to be confined to tropical and subtropical areas in the world, having been collected in Florida, Panama, and India. All 10 stocks belong to the group Sonneborn designated as *P. jenningsi*, syngen 1 (18).

P. multimicronucleatum, syngen 3. Stocks 12, 57, and 58 have EstA, $EstC_C$, and EstD esterases of identical mobility (Fig. 4). They appear to lack EstB and $EstC_A$ esterases. Stock 8MO has EstA, $EstC_C$, and EstD esterases that differ in mobility from the other three stocks (Fig. 4). It lacks $EstC_A$ but has an EstB esterase that migrates to a position similar to the slowest migrating A esterase (best seen in Fig. 6 in Ref. 4). The acid phosphatases show a pattern that is different from all other species and syngens of *Paramecium*, and all four stocks, including 8MO, have this pattern (see Fig. 2).

Table II shows the enzyme subtypes found in the four stocks of syngen 3. Identical phenotypes are observed in stocks 12, 57, and 58, with stock 8MO differing from the others in four of the five enzymes. Syngen 3 is confined to tropical areas, having been collected in Mexico, Puerto Rico, Costa Rica, and Thailand. It is interesting that the three stocks with identical phenotypes include stock 58 from Thailand.

P. multimicronucleatum, syngen 4. Both stocks 35 and 37 are identical in enzyme phenotype for EstA, EstB, $EstC_C$, and Acp (Fig. 4 and Table II). Both stocks lack $EstC_A$ esterases. The only difference between the stocks is the presence of a D esterase in

TABLE IV. *Mobilities of isozymes of each type of esterase in stocks of* Paramecium multimicronucleatum, *syngen 2.*

	Stocks																									
Mobility	4	9	11	17	18	20	25	42	43	44	46	48	49	P2-1	P2-3	P7-1	P7-2	P11-2	P11-3	P11-4	P16-1	P16-2	P16-3	B1A-2	B1A-6	B1A-7
5.1 cm[a]		?										✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4.8									✓																	
4.4	✓								✓																	
D subtype	I	III?							II			◂								III						▸
6.0									✓																	
5.8									✓																	
5.5	✓					✓	✓			✓																
5.2	✓					✓	✓			✓																
5.0			✓	✓	✓			✓			✓															
4.7	?					✓	?			✓																
4.5		✓	✓	✓	✓			✓			✓	✓														
4.2		✓										✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓
3.7													✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓
3.0																								✓	✓	✓
2.0																								✓	✓	
B subtype	II?	III	I	I	I	II	II?	I	IV	II	I	III	◂						V					VI	VI	VII
4.0	✓		✓	✓	✓	✓	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3.4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2.4																✓										
2.2		✓	✓						✓		✓															
1.5														✓		✓										
A subtype	I	III	II	I	I	I	I	I	II?	I	II	I	I	IV	I	V	I	I	I	I	I	I	I	I	I	I
2.1																										✓
1.4																				✓						
C_A subtype																				I						II
0.5																✓										
1.2																✓										
1.9			✓						✓												✓	✓	✓	✓	?	✓
3.0	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C_C subtype	I	I	Ia	I	I	I	I	I	Ib	I	I	I	I	I	I	II	I	I	I	I	Ia	Ia	Ia	Ia	Ia?	Ia

[a] Distance of bottom of band from origin.

stock 35 lacking in stock 37. Both stocks were collected from southern states (Florida and Texas) in the United States.

P. multimicronucleatum, syngen 2. In addition to the 13 stocks identified as belonging to syngen 2, 13 of the unidentified stocks could be assigned to this syngen. These included all of the stocks in the P series except for P3-1 and the three stocks from the B1A series. Table III lists all 26 stocks, giving the subtypes observed for six enzymes.

Immediately apparent is the extraordinary variability observed for the esterases among the 26 stocks. There are five EstA subtypes, seven EstB subtypes, four $EstC_C$ subtypes, and three EstD subtypes, with EstD missing from eight of the stocks. $EstC_A$ was only observed in two stocks. In contrast, all 24 stocks tested had similar Acp phenotypes. Some of the stock comparisons for the esterases are illustrated in the photographs: in previously identified stocks (Fig. 5) and in P and B1A stocks, compared to each other and to stock 49 or 46 (Fig. 6).

In examining esterase photographs it may not be apparent at first sight, but there is considerable sharing of isozymes between subtypes for EstA and between subtypes for EstB. This feature becomes more apparent in the display of mobilities shown in Table IV. Almost all stocks share two of the EstA isozymes, and the majority of stocks have only these two isozymes (subtype I). A third isozyme is shared by four of the stocks, a fourth by two of the stocks, and so on. The EstB isozymes are more variable among the stocks in terms of number of positions. Most stocks have two isozymes, the most common set (subtype V) found in stock 49, the 10 P stocks, and one of the B1A stocks (which has a third isozyme). A different set of two isozymes occurs in five other stocks (subtype I), one of the isozymes being shared with two other stocks (subtype III). Another set of two to three isozymes occurs in four other stocks (subtype II). The sharing of isozymes between subtypes is reminiscent of what was seen for the A esterases in *P. biaurelia,* where as few as two, but as many as seven, isozymes were observed in different stocks, with some of the isozymes being common to different subtypes (8).

Table III also gives the geographical origin of the stocks. All except one of the known syngen 2 stocks were collected in the United States. The exception, stock 49, was collected in Panama. The 10 new stocks from Panama and the three from Brazil are more similar in their esterases to stock 49 than any of the other known syngen 2 stocks. This is apparent from Fig. 6 and Table IV. The point is made even stronger in Table V, where the geographical distribution of all 26 stocks by esterase subtype is given. Of the 14 stocks collected in Panama or Brazil, 12 of them are EstA I, 11 are EstB V, seven are $EstC_C$ I, six are $EstC_C$ Ia (they have a second isozyme), and one is $EstC_C$ II. All 14 stocks are EstD III. The table also shows that a particular subtype is not confined to Panama or Brazil. For example, eight of the 20 stocks that were EstA I were collected from the United States.

Degree of variability. The frequency of variant stocks within a species or syngen is given in Table VI for each of the five esterases and acid phosphatase. In *P. jenningsi* none of the 10 stocks varied for EstA, EstB, $EstC_C$, $EstC_A$, or Acp. One of the stocks varied for EstD (lacking it). In *P. multimicronucleatum* syngen 2, 6/26 of the stocks had an EstA subtype other than

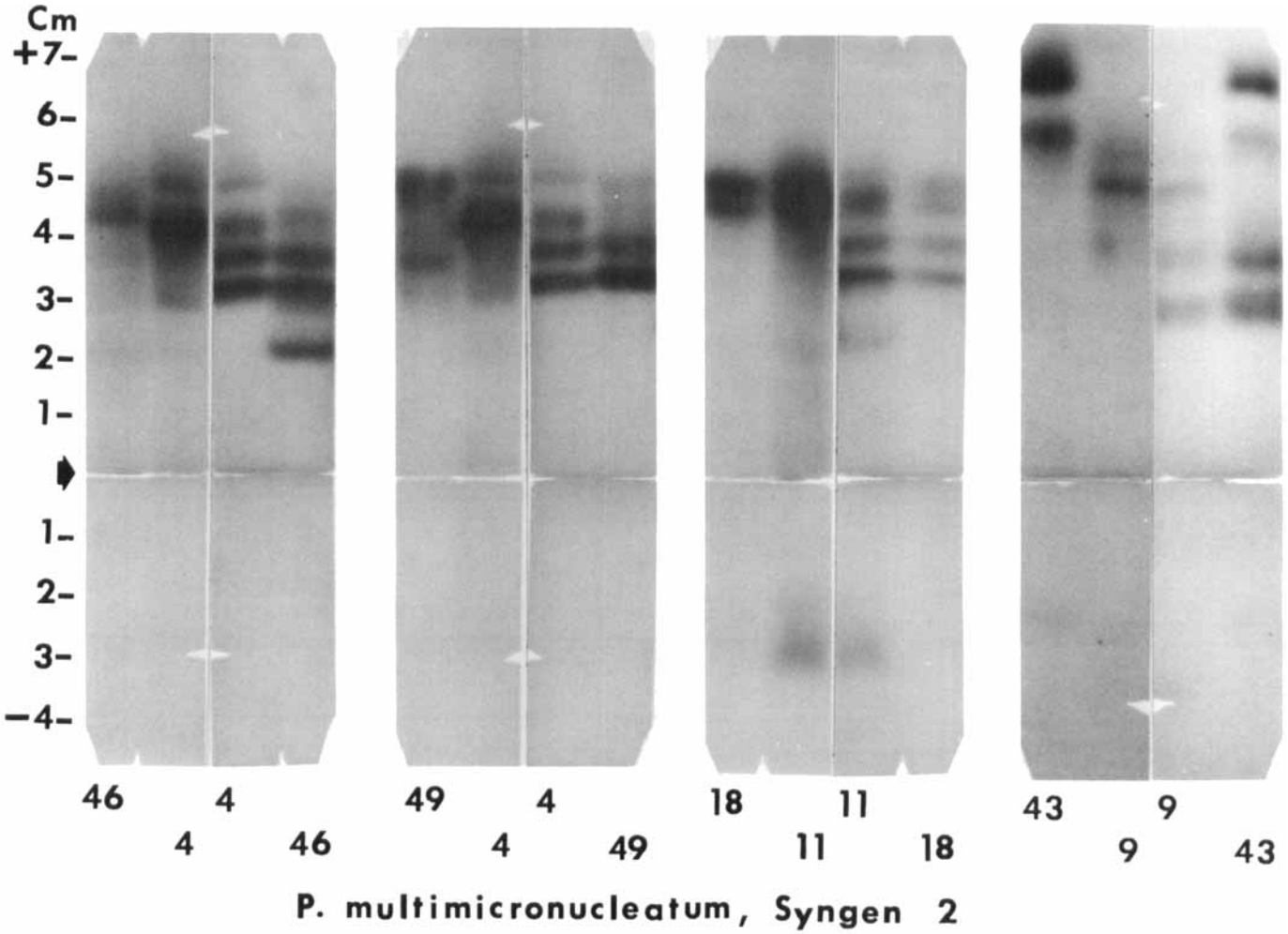

Fig. 5. Comparison of the A, B, C, and D esterases among stocks previously identified as belonging to *P. multimicronucleatum*, syngen 2. Substrate for the gel on *left:* α-naphthyl butyrate; on *right:* α-naphthyl propionate. The incubation time for the gels on the left was twice that for the gels on the right. See Fig. 1 for positions of the various types of esterases in stocks 46 and 49. $EstC_C$ is extremely faint in *P. multimicronucleatum*-2, except for certain stocks, such as stock 11.

subtype I, 2/26 had an $EstC_C$ subtype other than I or Ia, and of the 18 stocks that had EstD, 2/18 were subtype I or II (here the most frequent subtype was III). EstB was too variable to calculate frequencies, and none of the 24 stocks tested varied for Acp. In syngen 3, 1/4 stocks varied for EstA, EstB, $EstC_C$, and EstD. None of the four stocks had $EstC_A$ or varied for Acp. In syngen 4 only two stocks were examined, and only one difference was seen between them (for EstD).

TABLE V. *Geographical distribution of stocks in* Paramecium multimicronucleatum, *syngen 2, by esterase subtype.*

	Esterase A					Esterase B							Esterase C_C				Esterase D			
Origin	I	II	III	IV	V	I	II	III	IV	V	VI	VII	I	Ia	Ib	II	I	II	III	—
United States																				
Washington	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0
Massachusetts	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Pennsylvania	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0
Delaware	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Maryland	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Virginia	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
South Carolina	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Texas	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Mississippi	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Florida	2	1	0	0	0	1	1	0	1	0	0	0	2	0	1	0	1	1	0	1
Panama	9	0	0	1	1	0	0	0	0	11	0	0	7	3	0	1	0	0	11	0
Brazil	3	0	0	0	0	0	0	0	0	0	2	1	0	3	0	0	0	0	3	0
Totals	20	3	1	1	1	5	4	2	1	11	2	1	17	7	1	1	1	1	16	8

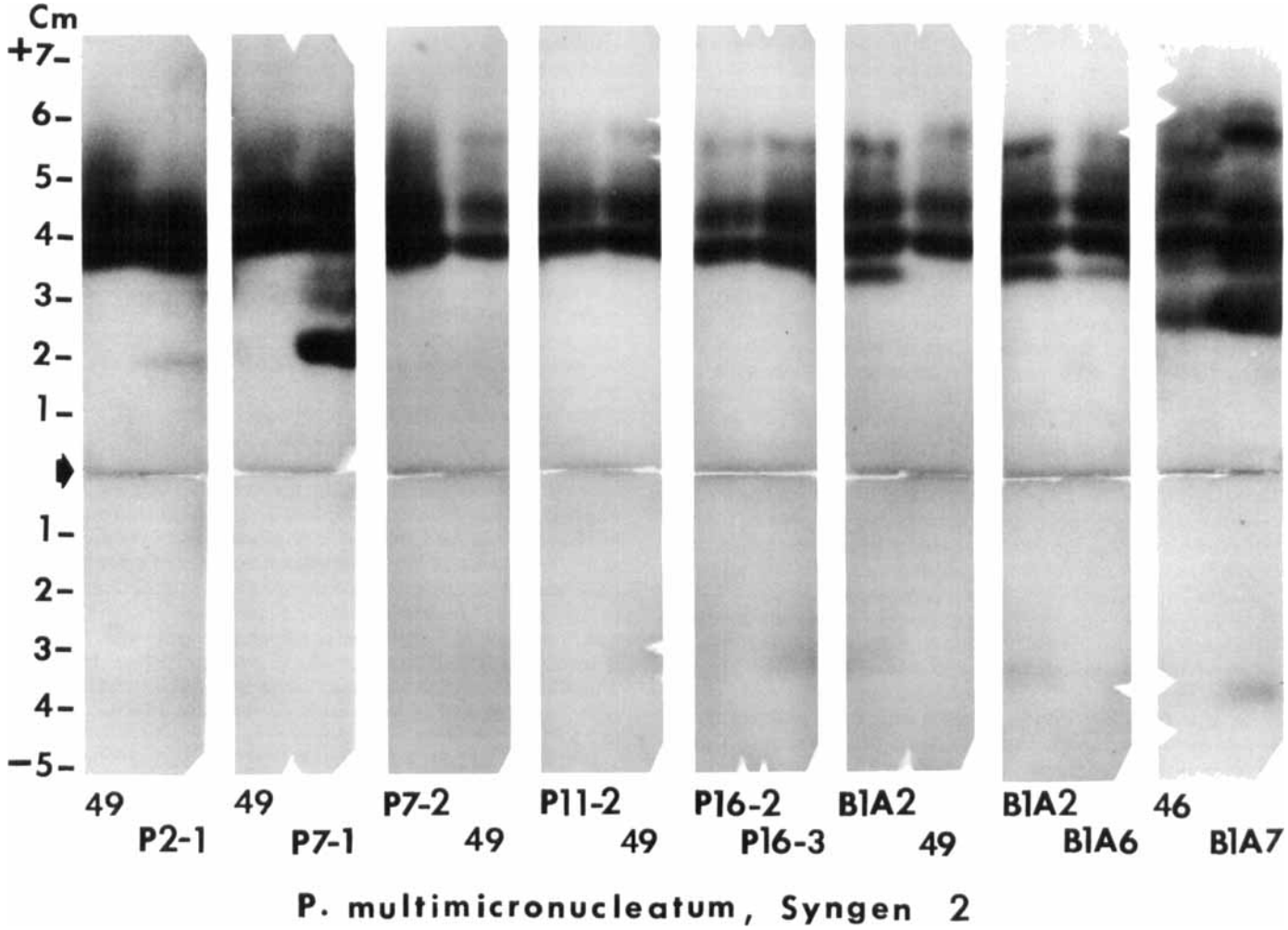

Fig. 6. Comparison of the A, B, C, and D esterases in stocks from the unidentified collections with known stocks (49 or 46) in *P. multimicronucleatum*, syngen 2. Substrate: α-naphthyl propionate. See Fig. 1 for positions of the various types of esterases in stocks 46 and 49. $EstC_C$ is extremely faint in *P. multimicronucleatum*-2, except for certain stocks, such as B1A7.

Variations for more than one enzyme occurred in two of the stocks in syngen 2 (three in stock 43 and two in P7-1). In syngen 3 stock 8MO varied for four of the five enzymes scored. The last column in Table VI includes stocks that varied for one, or more, of the five-six enzymes. The frequency of variant stocks in *P. jenningsi* (1/10) is similar to that observed in four species in the *P. aurelia* complex (26/188; Ref. 12). However, compared to the four *P. aurelia* species the frequency of variant stocks in syngens 2, 3, and 4 of *P. multimicronucleatum* (11/32) is significantly higher (Chi square = 5.85; df = 1; $P < 0.05$).

When the data on all 42 stocks are pooled, frequencies of 5.6–16.7% are observed for individual enzymes, with Acp showing

TABLE VI. *Frequency of variant stocks for five esterases and acid phosphatase in* Paramecium jenningsi *and in* P. multimicronucleatum, *syngens 2, 3, and 4.*

	Esterase A		Esterase B		Esterase C_C		Esterase C_A		Esterase D		Acid phosphatase		All 5–6 enzymes	
Species	Nos.[a]	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%
Paramecium jenningsi	0/10	0	0/10	0	0/10	0	0/10	0	1/10	10.0	0/10	0	1/10	10.0
P. multimicronucleatum														
Syngen 2	6/26	23.1	hypervariable		2/26	7.7	2/26	7.7	2/18	11.1	0/24	0	9/26	34.6
Syngen 3	1/4	25.0	1/4	25.0	1/4	25.0	—	—	1/4	25.0	0/4	0	1/4	25.0
Syngen 4	0/2	0	0/2	0	0/2	0	—	—	1/2	50.0	0/2	0	1/2	50.0
Total	7/32	21.9[b]	1/6	16.7	3/32	9.4	2/26	7.7	4/24	16.7	0/30	0	11/32	34.4
All stocks	7/42	16.7[b]	1/16	6.25	3/42	7.1	2/36	5.6	5/34	14.7	0/40	0	12/42	28.6

[a] Number of variant stocks/total number of stocks.
[b] 95% confidence limits: for 21.9 ± 14.3, for 16.7 ± 11.2. In the *aurelia* complex the frequency is 1.4 ± 1.9.

0%. The number of stocks available for screening was low; therefore, with one exception, none of the frequencies is significantly different from any of the frequencies observed for the same enzyme in species of the *aurelia* complex, where a much larger number of stocks was analyzed (12). The exception is the frequency of EstA, which is significantly higher in *P. multimicronucleatum* than in the *aurelia* complex (excluding *P. biaurelia,* which shows hypervariability for EstA).

DISCUSSION

Assignment of stocks to species. The enzyme electrophoresis procedure permitted stocks previously not identified as to species to be easily assigned to a particular species. Of the 18 stocks tested, all of the S series collected in India and one of the P series collected in Panama were identified as belonging to *P. jenningsi,* and ten of the P series and all of the B1A series collected in Brazil could be assigned to syngen 2 of *P. multimicronucleatum* on the basis of similarity of enzyme phenotypes. On the basis of morphological criteria, these assignments were confirmed by Daggett and Nerad of the American Type Culture Collection (personal communication). Inclusion of these stocks doubled the numbers available in these two species, thereby facilitating examination of intraspecies variation and comparison with members of the *aurelia* complex.

Phenotypes of variants. Variant stocks were observed in all of the species examined here. In some cases the variant may lack the enzyme, as observed for EstD in one stock of *P. jenningsi* and in syngen 4 of *P. multimicronucleatum.* In most cases a change in mobility of the electrophoretic form of the enzyme is seen. In the *P. aurelia* complex many of the esterase variants have a mobility characteristic of a common subtype in another species of the complex (8). In syngen 3 of *P. multimicronucleatum* the EstA isozymes in stocks 12, 57, and 58 are similar in mobility to the subtype commonly observed in *P. tetraurelia* and *P. octaurelia,* while those in stock 8MO are similar in mobility to the common subtype shared by *P. primaurelia, P. triaurelia, P. pentaurelia, P. septaurelia, P. undecaurelia, P. tredecaurelia,* and *P. quadecaurelia* (13). The $EstC_C$ in 8MO is similar to the common subtype shared by *P. septaurelia, P. undecaurelia,* and *P. tredecaurelia.*

In some cases unique phenotypes are observed for the variant stocks. This was observed for the esterase variants seen in *P. multimicronucleatum,* syngen 2, none of which were similar in mobility to subtypes found in other species. This was also the usual situation in *P. biaurelia* (8). Hypervariability for EstB was observed in syngen 2 of *P. multimicronucleatum,* where, in addition, several unique subtypes were found for EstA, $EstC_C$, and EstD. Hypervariability for EstA and $EstC_A$ was observed in *P. biaurelia* (12). Thus, in these two species—one from the *aurelia* complex, the other from the *multimicronucleatum* complex—we see unique phenotypes for the esterase variants and hypervariability.

Clustering of variations. Ten of the 12 variant stocks occurred in syngens 2 and 3 of *P. multimicronucleatum.* Of these 10 stocks, three had multiple variations. One varied for two enzymes, one varied for three enzymes, and one varied for four enzymes. Clustered variations were also observed in four species of the *P. aurelia* complex (12), where 10 of 26 stocks had multiple variations. Where clustering occurs, the potential for misclassification of stocks becomes a problem, especially where only a small number of enzymes is sampled. In the case of stock 8MO in *P. multimicronucleatum*-3 that varied for four different enzymes, its Acp phenotype uniquely placed it in the correct species.

Geographical differentiation. One of the outstanding and unique features of the *P. aurelia* complex was the virtual absence of geographical differentiation between stocks of any one species with regard to enzyme variation. Stocks that were similar in phenotype, whether they had the common or a variant enzyme phenotype, were collected from sites widely separated geographically (2, 12). In *P. jenningsi* (10 stocks) and *P. multimicronucleatum*-3 (four stocks), the common enzyme phenotypes are widely distributed geographically; however, there are far too few stocks to decide if geographical differentiation is absent. In *P. multimicronucleatum*-2, on the other hand, some geographical differentiation does seem to occur, since the 13 newly identified stocks from Panama and Brazil are more similar in their esterases to stock 49 from Panama than the other 12 stocks collected in the United States. Thus, lack of geographical differentiation may not always occur in species of *Paramecium. P. multimicronucleatum*-2 is the only species of those examined here that does not undergo autogamy. Possibly this difference in breeding behavior permits a greater degree of geographical differentiation.

Frequency of intraspecies variation. The frequency of intraspecies variation was low in *P. primaurelia, P. biaurelia, P. tetraurelia,* and *P. octaurelia* for EstB, $EstC_C$, and Acp, and in *P. primaurelia, P. tetraurelia,* and *P. octaurelia* for EstA, and in *P. octaurelia* for $EstC_A$ (12). *P. biaurelia* showed hypervariability for EstA and $EstC_A$ and unique phenotypes for the other enzymes. Thus, the level of intraspecies variation appeared to be higher for *P. biaurelia* than for *P. primaurelia, P. tetraurelia,* and *P. octaurelia.* The frequency of variant stocks in *P. jenningsi* is similar to that observed in the *P. aurelia* species. However, a significantly higher frequency of variant stocks occurs in syngens 2, 3, and 4 of *P. multimicronucleatum*—34.4% compared to 13.8%.

In the *P. aurelia* species complex, differences in the frequency of intraspecies variation were observed between enzymes (12). EstA, EstB, $EstC_C$, $EstC_A$, and Acp had frequencies ranging between 1.4 and 6.9%. For the same enzymes in *P. multimicronucleatum,* the frequencies vary between zero (Acp) and 21.9% (EstA); however, only the frequency for EstA is significantly different from the frequency observed for the *P. aurelia* species.

Less well known biologically and genetically are the syngens of *P. multimicronucleatum* (16–18, 21). Syngen 2 has been examined genetically, and it is known to have a remarkable mating type system in which a cyclical switch-over in mating type occurs during a 24-hour period in response to exposure to light and dark (20). Autogamy does not occur in this syngen (17). The lack of autogamy and presence of an immature period led Nyberg (15) to characterize syngen 2 as an outbreeder. He found higher tolerance to environmental stress in this syngen compared to inbreeding species such as *P. tetraurelia.* In addition, he found more variability among stocks of the inbreeding species. These observations supported his thesis that inbreeding is an adaptation that enables closer genetic tracking of environmental changes. However, we did not find less variability for the enzymes in the outbreeding *P. aurelia* species, such as *P. biaurelia,* as expected; rather, we found more variability in this species (12). *P. multimicronucleatum*-2 is even more of an outbreeder than *P. biaurelia.* The level of enzyme variation appears to be even greater, since most enzyme phenotypes are unique and hypervariability occurs for some of the enzymes. This syngen (species) also seems to exhibit some geographical differentiation unlike the species in the *aurelia* complex, including *P. biaurelia.* Explanation for some of these differences should be followed up by future studies on the genetics, breeding system, and ecology of this interesting species.

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Titles of Recent Books Received for Review

Following the custom initiated in the February 1981 number of our *Journal of Protozoology*, we are presenting here the titles of books of protozoological interest that have been received in recent weeks by the Managing Editor for possible review in the subsequent issues. [Publishers are reminded that copies of their latest works in areas appropriate for our readership may be sent to us without specific solicitation on our part if both quick listing and likely later full review treatment in sections of this *Journal* are desired.]

Amos, W. B. & Duckett, J. G., eds. 1982. *Prokaryotic and Eukaryotic Flagella.* (*Symp. Soc. Exp. Biol.*, Vol. 35.) Cambridge University Press, New York. 632 pp. $79.50.

Asher, Ann & Spalding, D. F., eds. 1982. *Culture Centre of Algae and Protozoa: List of Strains 1982.* Institute of Terrestrial Ecology, Natural Environment Research Council, Cambridge, England. 100 pp.

Baker, John R. 1982. *Parasitic Protozoa in British Wild Animals.* Institute of Terrestrial Ecology, Natural Environment Research Council, Cambridge, England. 24 pp. £1.70.

Baker, John R. 1982. *Perspectives in Trypanosomiasis.* (Proc. 21st Trypanosomiasis Seminar, London, Sept. 1981.) John Wiley & Sons, New York. 105 pp. $29.95.

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