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PROTEIN BAND DISTRIBUTION IN THREE SPECIES OF PLANARIANS USING ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GELS

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ABSTRACT

A crude protein extract from *Phagocata gracilis, Phagocata velata,* and *Dugesia dorotocephala* was prepared for isoelectric focusing in polyacrylamide gels by grinding, sonic treatment, and centrifugation. The protein banding due to electrophoresis supports the morphological evidence for distinguishing these three groups of organisms as separate species.

INTRODUCTION

Chemotaxonomy has been widely used to support traditional methods of taxonomy to show relationships between closely related groups. Most of these studies have involved electrophoresis of protein from tissues and various fluids. Peter (1971) examined the systematic grouping of six species of planarians using zone electrophoresis. He found that comparisons between different populations of a given species yielded higher degree of similarity than those between different species. Individuals as well as populations could be distinguished by their protein spectra, but the varying interspecific correspondences could not be used for taxonomic grouping. Bell and Grubbs (1969) analyzed four species of planarians utilizing cellulose polyacetate electrophoresis. They found that similarities in the protein patterns tended to verify existing taxonomic relationships.

Nixon and Taylor (1977) examined the patterns of genetic differentiation based on protein difference in 22 populations of *Polycelis coronata* and one population of *Dugesia tigrina* using polyacrylamide disc gel electrophoresis. Their results suggested different rates of evolution due to lack of morphological variation which did not correspond with the biochemical divergence.

The purpose of this study was to examine protein differences between three species of freshwater planarians: *Phagocata gracilis, Phagocata velata,* and *Dugesia dorotocephala* using isoelectric focusing in polyacrylamide gels.

METHOD AND MATERIALS

Three species of freshwater planarians (platyhelminthes, Turbellaria, Tricladida, Paludicola) were collected in the central area of Tennessee: *Phagocata gracilis* (Haldeman), *Phagocata velata* (Stringer), and *Dugesia dorotocephala* (Woodworth). *Phagocata gracilis* was collected from many small spring-fed streams below Center Hill Dam in an abandoned limestone quarry. The streams were located one-half kilometer west of the dam on State Route 141 in Dekalb County. *Phagocata velata* was collected from a wet weather stream in Wilson County. The stream was located two kilometers west of Leeville on the E. N. Brown farm approximately 615 meters north from Hickory Ridge Road. *Dugesia dorotocephala* was collected in a creeek below an artificially maintained trout farm in Hickman County. The creek runs below I-40 (west) at Bucksnort, Tennessee.

Planarians were divided into five groups each containing 20 individuals approximately the same size. The worms were ruptured ultrasonically, and the sample centrifuged at 54,333xg for fifteeen minutes in a Beckman (Model L3-50) Ultracentrifuge at a rotor temperature of 5°C. The supernantant containing the protein was frozen, and concentrated on a Thermovac Freezer-dryer. The powdered protein sample was weighed and the sample solubilized in 50% sucrose in 0.0375M tris sulfate buffer. The sample solution was stored at—55°C until assayed. Electrofocusing gels containing carrier ampholine (pH 3-10) were prepared by the method of Ortec (1973). After polymerization each gel tube received 15 ml of sample for isoelectric focusing in a Buchler electrophoretic system. The cathodal solution contained 0.15M refrigerated ethanolamine. Refrigerated 0.1N HCl was used for the anodal solution. During electrophoresis the buffer temperature of the lower tank was maintained at 9.5°C. The Ortec (Model 4100) pulsed constant power supply was used to regulate the voltage and frequency length of the pulsed current. The sample proteins migrated in the direction of the anode to isoelectric points. The pulsed constant power supply setting for the cathode to anode sample migration was 50 v with a pulse rate of 100 pulses per second with a two cell capacitance of 1.0 uF. The current was applied until milliampherage dropped to a minimum and no further migration was apparent.

The gels were fixed in 10% trichloroacetic acid (TC) for 30 minutes. The gel stain was prepared by making a 0.1% solution of Coomassie Brilliant Blue in 15% TCA. This solution was filtered and diluted 1-30 using 15% TCA. Each gel was placed in 25 ml of stain. The gels were stained for 20 minutes at 65°C or until the desired staining results were obtained. If destaining was necessary excess stain was removed by two 35 minute washings in 45% methanol-10% acetic acid. The gels were stored in a 70% acetic acid solution. Protein determinations were by the method of Lowry *et. al.* (1951).

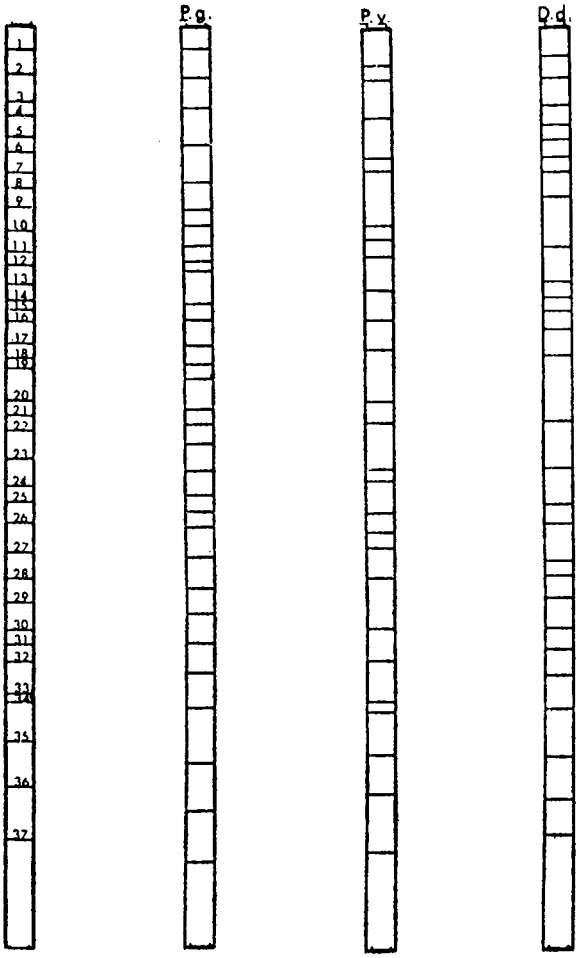

FIG. 1. *Protein bands of* P. gracilis, P. velata, *and* D. dorotocephala.

Results and Discussion

Examination of the protein patterns of the three species of planarians revealed considerable variation in density and electrophoretic mobility. Location of protein band positions in the three species of planarians was variable (Fig 1). The combined samples of *P. gracilis, P. velata* and *D. dorotocephala* contained 37 protein bands. *Phagocata gracilis* contained 31 bands with bands 4, 6, 8, 15, 31, and 34 absent. *Phagocata velata* contained 26 bands with bands 4, 6, 8, 12, 14, 16, 18, 19, 22, 29 and 32 absent. *D. dorotocephala* contained 28 bands with bands 9, 11, 13, 18, 19, 21, 22, 25 and 34 absent. Common bands absent in *P. gracilis* and *P. velata* were 4, 6 and 8; *P. gracilis* and *D. dorotocephala* band 34; and *P. velata* and *D. dorotocephala* bands 18, 19 and 22.

The total protein concentrations were determined for three species of planarians which had been divided into five groups of 20 worms. The mean concentration was: 2.24 mg $\pm$ 0.22 for *P. gracilis,* 2.00 mg $\pm$ 0.07 for *P. velata* and 2.45 mg $\pm$ 0.05 for *D. dorotocephala.* No significant differences (*t* test) were apparent between the mean protein concentrations of the three species of planarians. However, when protein band concentrations were compared, significant differences were noted although there was no pattern which suggested similarity between these taxonomic groups (Table I).

Based on these results isoelectric focusing may serve as a supplemental procedure for examining taxonomic difference between these three species of planarians.

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TABLE I: *Protein bands in three species of planarians following separation by isoelectric focusing. Values are expressed as mean micrograms protein ± SE for five groups of 20 worms each.*

Band	P. gracilis	P. velata	D. dorotocephala	Pg vs. Pv[a]	Pg vs. Dd[a]	Pv vs. Dd[a]
1	60.32± 7.86	139.09±22.75	129.19±21.10	*		
2	45.48±13.39	62.26±11.59	69.29± 8.81			
3	38.89±16.86	156.62±36.21	53.02±10.71	*	*	
4	ND	ND	30.13± 3.50			
5	48.66±19.14	109.14±17.77	28.68± 3.30		**	
6	ND	ND	25.21± 3.29			
7	49.86±13.69	26.10± 4.38	18.72± 2.84			
8	ND	ND	32.43± 1.44			
9	23.55± 4.93	78.23±13.33	ND	*		
10	12.85± 2.82	19.29± 2.77	57.31± 7.29		**	**
11	21.33± 5.95	23.00± 2.81	ND			
12	16.03± 5.02	ND	35.51± 5.91			
13	13.67± 4.29	53.77± 5.96	ND	**		
14	41.66±12.58	ND	33.51± 4.73			
15	ND	68.57±10.02	28.75± 4.49			*
16	25.57± 9.24	ND	34.95± 7.15			
17	55.54±19.35	95.32± 9.53	51.71± 7.02			
18	39.32±11.09	ND	ND			
19	43.49± 8.76	ND	ND			
20	95.64±23.42	225.10±15.10	349.17±25.97	**	**	**
21	49.14±12.86	88.32± 6.74	ND	**		
22	58.85± 9.59	ND	ND			
23	74.80±15.57	191.99±17.11	250.81±23.01	**	**	
24	60.83±13.27	32.41± 3.92	125.25±12.07		**	**
25	35.20± 8.11	102.76±14.54	ND	*		
26	33.67± 5.03	63.93± 7.91	49.00±13.07	*		
27	67.40±12.73	53.27± 3.99	117.36± 9.40		*	**
28	76.90±11.38	85.60± 9.77	46.52± 6.75			
29	63.27±10.29	ND	73.81± 4.30			
30	95.28±14.77	111.96± 9.43	121.13±11.39			
31	ND	113.42±14.34	102.58±13.50			
32	103.52±18.15	ND	263.96±64.46			
33	167.72±34.89	133.73±10.62	139.77±81.71			
34	ND	47.88± 5.56	ND			
35	260.24±34.60	215.96± 7.53	192.99±17.80			
36	151.81±47.30	166.98±12.50	147.78±15.18			
37	36.85±16.80	129.12±18.97	50.96± 7.47	*	*	

[a]Statistical difference at the 0.01 (**) or 0.05 (*) level of confidence as determined by the Duncan's Multiple Range Test.

FORMER OAK RIDGE SCIENTIST AND JTAS EDITOR DIES DURING 1980

Helen Lavina Ward, 69, formerly a scientific analyst in biology with the Oak Ridge Technical Information Center from 1962 to 1979 and editor of JTAS from 1954 to 1964, died August 22, 1980 in Americana Healthcare Center in Lafayette, Indiana where she had been a patient since April. She is survived by three sisters and one brother who currently reside in Indiana, Florida and Arizona.