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Biochemical Identification of *Phlebotomus (Larroussius) pedifer* and *Phlebotomous (Larroussius) elegonensis*

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Key Word Index—*Phlebotomus pedifer*; *P. elegonensis*; electrophoresis; thin-layer starch gel electrophoresis; isoelectric focusing.

Abstract—Females of the vector of *Leishmania aethiopica*, *Phlebotomus pedifer*, have previously been morphologically indistinguishable from the non-vector, *P. elegonensis*. The present studies have biochemically separated these two species. Differences were observed in the mobility of four enzymes, GPI, HK, ICD and PGM on thin-layer starch gel electrophoresis. Using the isoelectric focusing technique, the two species could be differentiated by one enzyme, GPI.

Introduction

Dipteran sibling species, comprising morphologically indistinguishable populations that are reproductively isolated, are usually very easily distinguished by many biochemical differences as in *Anopheles* [1, 2], *Aedes* [3, 4] and *Simulium* [5]. In studies on the subfamily Phlebotominae, biochemical methods have also been applied [6–8].

The present studies set out to use biochemical techniques of thin-layer starch gel electrophoresis and isoelectric focusing on agarose to differentiate *Phlebotomus pedifer*, a vector of *Leishmania aethiopica* [9], from *P. elegonensis* (non-vector) whose females are morphologically identical. Previously the only clue to their identity has been small differences in their male terminalia and the females could only be differentiated by rearing them individually in isolation or by collecting them in copula. Since the females of *P. pedifer* are involved in disease transmission, their identity should be verified.

Results and Discussion

Of the 14 enzymes assayed in thin-layer starch gel electrophoresis, nine; glucose phosphate isomerase (GPI), hexokinase (HK), isocitric dehydrogenase (ICD), malate dehydrogenase (MDH), malic enzyme (ME), mannose phosphate isomerase (MPI), phosphoglucomutase (PGM) and L-threonine-3-dehydrogenase (TDH) could be detected in individual sandflies with varying degrees of intensity. The profiles of six of these enzymes which were fairly distinct and reproducible are presented (Fig. 1). The frequency of successful identification is shown in Table 1. Phosphoglucomutase is not presented in Fig. 1 because it had two or three different allelic combinations in both *P. pedifer* and *P. elegonensis* during every run. These alleles expressed themselves in different combinations in each of these species at every run. In each PGM run, the most common allele of *P. elegonensis* always had a lower mobility than the most common allele of *P. pedifer*. TDH was too faint and MPI too inconsistent and are therefore not presented. Aconitate hydratase (ACON), alcohol dehydrogenase (ADH), glucose-6-phosphate dehydrogenase (G-6-PD), nucleosidase hydralase (NH) and superoxide dismutase (SOD) could not be detected in individual sandflies. The enzymes extracted from a male or female of each species had identical mobilities.

It is evident that GPI, HK, ICD and PGM can differentiate *P. pedifer* from *P. elegonensis*. For each of these enzymes *P. elegonensis* had a lower mobility than *P. pedifer*.

Using isoelectric focusing on agarose, only five of the 14 enzymes assayed could be detected in individual sandflies. These were GPI, HK, MDH, ME and PGM. With this method, only GPI differentiated *P. pedifer* from *P. elegonensis*.

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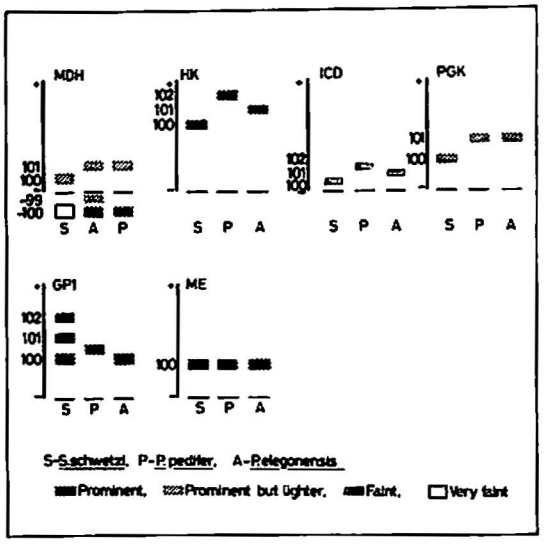

FIG. 1. THIN-LAYER STARCH GEL ELECTROPHORESIS. Enzyme profiles of MDH, HK, ICD, PGK, GPI and ME for *P. pedifer* and *P. elegonensis*. *Sergentomyia schwetzi* was used as a marker species.

P. pedifer had only four distinct bands whereas *P. elegonensis* had six distinct bands for GPI (Fig. 2). The frequency of successful identification is shown in Table 2. Hexokinase, MDH, ME and PGM could not differentiate the two species (Fig. 2).

When the two biochemical techniques utilized during this study were compared, the thin-layer starch gel electrophoresis technique was more diagnostic than isoelectric focusing for differentiating *P. pedifer* from *P. elegonensis*. With the former method, the two species could be separated by four enzymes, GPI, HK, ICD and PGM, with an electromorph frequency of 1.00 for the most common allele, while in the latter method separation was possible only by one enzyme, GPI, also with an electromorph frequency of 1.00 for the most common allele. It was further observed that the IEF technique revealed more isoenzyme bands than the thin-layer starch gel technique for the enzymes, GPI and PGM.

Righetti and Drysdale [10] discussed the possible reasons for an increased number of bands revealed by IEF and concluded that they may be a result of deamination, deacetylation

TABLE 1. RELATIVE MIGRATION OF BANDS FOR THIN-LAYER STARCH GEL ELECTROPHORESIS FROM THE ORIGIN

Enzyme	Number of specimens			Number of trials	Enzyme electromorph designation in parentheses and frequency		
	S. shwetzi	*P. pedifer*	*P. elegonensis*		*S. shwetzi*	*P. pedifer*	*P. elegonensis*
ACON	6	16	18	4	Nil	Nil	Nil
ADH	4	19	16	4	Nil	Nil	Nil
G-6-PD	4	8	11	3	Nil	Nil	Nil
GPI	10	39	37	10	$(100)^{1.00}$	$(100.5)^{1.00}$	$(100)^{1.00}$
					$(101)^{1.00}$		
					$(102)^{1.00}$		
HK	10	29	26	5	$(100)^{1.00}$	$(102)^{1.00}$	$(101)^{1.00}$
ICD	5	17	16	8	$(100)^{1.00}$	$(102)^{1.00}$	$(101)^{1.00}$
MDH	6	19	18	9	$(100)^{0.83}$	$(101)^{0.89}$	$(101)^{0.89}$
					$(-100)^{0.89}$	$(-100)^{0.89}$	$(-99)^{0.11}$
ME	5	22	19	7	$(100)^{1.00}$	$(100)^{1.00}$	$(100)^{1.00}$
MPI	1	5	5	3	$(100)^{0.20}$	$(100)^{0.20}$	$(100)^{0.20}$
					$(101)^{0.20}$	$(101)^{0.20}$	$(101)^{0.20}$
					$(102)^{0.20}$	$(102)^{0.20}$	$(102)^{0.20}$
NH_2	2	8	9	2	Nil	Nil	Nil
PGM	8	25	27	8	$(100)^{0.00}$	$(95)^{0.50}$	$(90)^{0.13}$
					$(101)^{0.40}$	$(98)^{0.50}$	$(95)^{1.00}$
					$(102)^{0.40}$	$(100)^{1.00}$	$(100)^{0.50}$
						$(101)^{0.50}$	$(101)^{0.13}$
PGK	4	10	9	3	$(100)^{1.00}$	$(101)^{1.00}$	$(101)^{1.00}$
SOD	2	10	9	2	Nil	Nil	Nil
TDH	7	19	18	4	$(100)^{1.00}$	$(100)^{1.00}$	$(100)^{1.00}$

The numerical values designated for each electromorph are arbitrary. The lowest anodic electromorph for the control species (*S. shwetzi*) is designated 100, subsequent electromorphs are designated 101, 102, 103 etc. Similarly the lowest cathodic electromorph for the control was designated −100. Electromorphs for each species being studied are relative to that of the control species (*S. shwetzi*) (refer also to Fig. 1).

TABLE 2. RELATIVE MIGRATION OF BANDS FOR ISOELECTRIC FOCUSING

Enzyme	Number of specimens			Number of trials	Enzyme electromorph designation in parentheses and frequency		
	S. shwetzi	*P. pedifer*	*P. elegonensis*		*S. shwetzi*	*P. pedifer*	*P. elegonensis*
ACON	1	5	4	1	Nil	Nil	Nil
ADH	1	4	5	1	Nil	Nil	Nil
G-6-PD	2	7	10	2	Nil	Nil	Nil
GPI	5	19	19	5	(100)$^{1.00}$	(104)$^{1.00}$	(101)$^{1.00}$
					(103)$^{1.00}$	(105)$^{1.00}$	(102)$^{1.00}$
					(104)$^{1.00}$	(106)$^{1.00}$	(103)$^{1.00}$
					(105)$^{1.00}$	(107)$^{1.00}$	(104)$^{1.00}$
					(106)$^{1.00}$		(105)$^{1.00}$
					(107)$^{1.00}$		(106)$^{1.00}$
HK	7	25	37	7	(100)$^{1.00}$	(101)$^{1.00}$	(101)$^{1.00}$
ICD	4	20	20	6	Nil	Nil	Nil
MDH	4	16	16	4	(100)$^{1.00}$	(100)$^{1.00}$	(100)$^{1.00}$
					(−100)$^{1.00}$	(−100)$^{1.00}$	(−100)$^{1.00}$
ME	5	20	19	5	(100)$^{1.00}$	(100)$^{1.00}$	(100)$^{1.00}$
MPI	8	31	32	7	Nil	Nil	Nil
NH_2	1	5	4	1	Nil	Nil	Nil
PGM	5	22	26	7	(100)$^{1.00}$	(100)$^{1.00}$	(100)$^{1.00}$
					(101)$^{1.00}$	(101)$^{1.00}$	(101)$^{1.00}$
					(102)$^{1.00}$	(102)$^{1.00}$	(102)$^{1.00}$
					(103)$^{1.00}$	(103)$^{1.00}$	(103)$^{1.00}$
					(104)$^{1.00}$		
PGK	2	8	7	2	Nil	Nil	Nil
SOD	2	9	10	2	Nil	Nil	Nil
TDH	2	12	8	2	Nil	Nil	Nil

The numerical values designated for each electromorph are arbitrary. The lowest anodic electromorph for the control species (*S. shwetzi*) are designated 100, subsequent electromorphs are designated 101, 102, 103 etc. Similarly the lowest cathodic electromorph for the control species was designated −100. Electromorphs for each species being studied are relative to that of the control species.

and different pH's are used or that their relative quantities in these specimens were below detection levels by the techniques used.

Comparison of *P. pedifer* and *P. elegonensis* using biochemical methods should be extended to other areas where these two species occur sympatrically in Kenya (Lake Naivasha area) and outside Kenya (Ethiopia). Such studies should also include *P. longipes*, which although not found in Kenya, has a female that is morphologically similar to the female of *P. pedifer* and *P. elegonensis*. *Phlebotomus longipes* is economically important because it is the vector of *L. tropica* which causes dermal leishmaniasis in Ethiopia [12].

With the revelation that *P. elegonensis* is different from *P. pedifer*, it is important to investigate its possible involvement in the transmission of cutaneous leishmaniasis. Thus this work should be valuable in epidemiological studies trying to pinpoint the vector of cutaneous leishmaniasis due to *L. aethiopica*.

Experimental

Thin-layer starch gel electrophoresis. Single insectary-reared sandfly homogenates were subjected to electrophoresis in a buffer system, on 10% electrostarch 1 mm thick gels. The specimens used were not directly from the field because the identity of the females had to be ascertained by rearing them in isolation and obtaining their identity by the identity of their sons. These were then representative of field material because specimens beyond the second generation were not used, thus eliminating the problem of inbreeding. Tank and developer buffers and staining conditions were as in [6].

Isoelectric focusing. Sandfly homogenates prepared as with thin-layer starch gel electrophoresis were subjected to isoelectric focusing on 114×225×1 mm agarose IEF. Developer buffers and staining conditions were as above.

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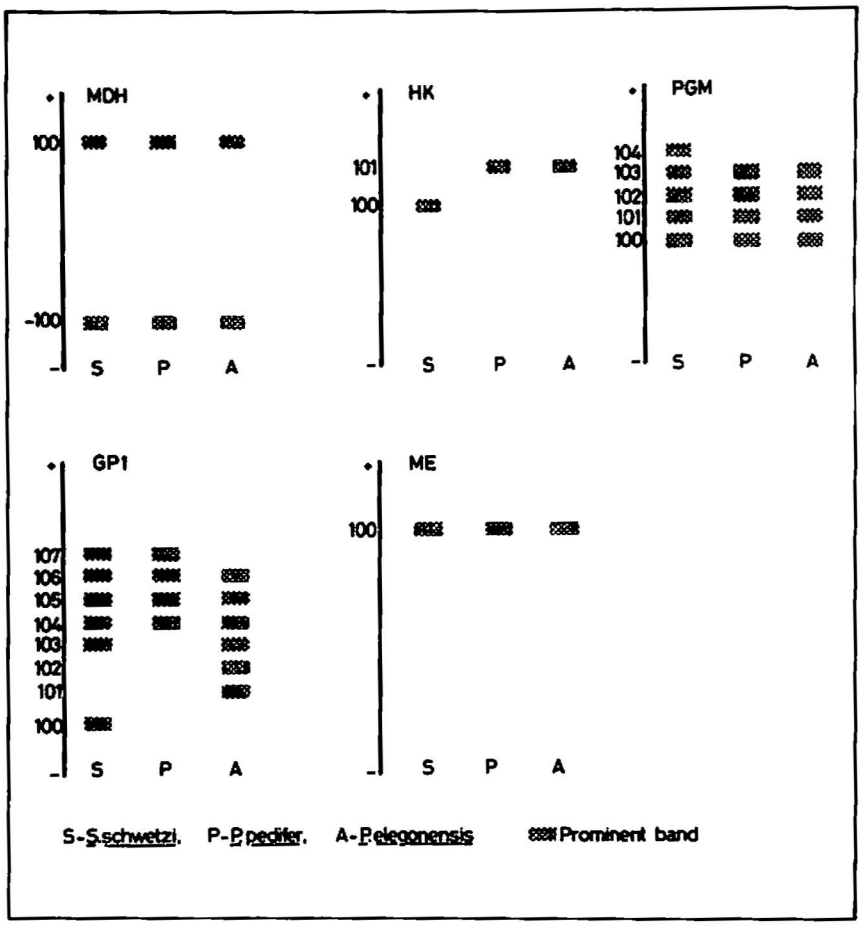

FIG. 2. ISOELECTRIC FOCUSING ON AGAROSE. Enzyme profiles for MDH, HK, PGM, GPI and ME for *P. pedifer* and *P. elegonensis*. *Sergentomyia schwetzi* was used as a marker species.

and interactions with ligand-like substrates or co-factors after translation. It is most likely that the increased number of bands was not revealed with enzymes MDH, HK and ME which were more or less similar to bands resolved by thin-layer starch gel electrophoresis, because the different pH ranges used in the present investigation were not the optimum pH's for the enzymes as they gave results contrary to those of some workers; for example, the work of Allsopp and Gibson [11] who compared the two biochemical techniques using the enzymes, LDH and GPI, from several parasites, but preferred isoelectric focusing because it was more discriminatory.

But whatever biochemical technique used, *P. pedifer* could be differentiated from *P. elegonensis*. These investigations agree with those of Ward *et al.* [7] and Petersen [8] that biochemical methods are reliable and may be used to differentiate between closely related species of sandfly groups. These biochemical investigations confirm the work of Miles and Ward [6] that the main components of the enzymes detected are the same for male and female. The demonstration that ADH, G-6-PD, NH and SOD could not be detected in individual sandflies is not necessarily an indication that these enzymes were absent. It is possible that these enzymes may be detected if other buffers

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