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ISOZYME VARIATION IN *SIMULIUM (EDWARDSELLUM) DAMNOSUM* S.L. (DIPTERA: SIMULIIDAE) FROM KENYA

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ABSTRACT. Isozyme variation of the *Simulium damnosum* sibling species complex was studied by cellulose acetate electrophoresis (CAE) from four Kenyan river systems. Two enzymes, PGM and HK, were diagnostic and differentiated the larvae collected in Western and Nyanza provinces from the larvae collected at Mt. Kenya. Allele frequency differences of the enzyme PGI allowed about 75% separation of the geographically distinct populations.

INTRODUCTION

In recent years, isoenzyme electrophoresis has been extensively used in the discrimination and identification of medically important insect sibling species including Simuliidae, (Coker 1973, May et al. 1977, Meredith and Townson 1981, Snyder 1982), *Aedes aegypti* (Linn.) (Tabachnick et al. 1979, Powell et al. 1980), *Culex pipiens* Linn. (Miles and Patterson 1979), *Lutzomyia* and *Phlebotomus* (Petersen 1980, Ward et al. 1981) and *Anopheles gambiae* Giles (Miles 1979).

Raybould and White (1979) indicated that there are four members of the *Simulium damnosum* sibling species complex in Kenya. These four members have been classified by Dunbar (1976) into two major groups; the Sanje group and the Kibwezi group. The Sanje group, which is found only in eastern Africa, is a group of eight forms and a ninth form which is a hybrid of Sebwe and Nyamagasani forms. Two of these, the 'Nkusi' and the 'Kisiwani' forms are found in Kenya. The 'Nkusi' form is found only in western Kenya while the 'Kisiwani' is found in the Mt. Kenya area (Carlsson 1970).

The Kibwezi group, which is also only reported from eastern Africa to date contains two forms: the 'Kibwezi' and the 'Mutonga' forms. The former has been reported from both Kenya and Tanzania, from the Kibwezi River and the Pangani River respectively. The 'Mutonga' form has only been collected from the eastern slopes of Mt. Kenya (Dunbar 1976). Six perennial river-systems have been found to contain *S. damnosum* s.l. breeding habitats in western Kenya and around Mt. Kenya. These rivers are the Thiba and the Nyamindi, draining the eastern slopes of Mt. Kenya, and the Yala, Lusumu, Isiukhu and the Nzoia, draining the Nandi Hills escarpment and the Mt. Elgon areas.

Previous isozyme studies (Mebrahtu et al. 1986) on the *S. damnosum* sibling species complex from Kenyan breeding sites revealed that there is a significant difference in hexokinase (HK) allele between those species breeding in the Lusumu River and those breeding in the Nzoia River. Similarly, geographic variation in the PGI allele was also highly significant between *S. damnosum* s.l. breeding in Lusumu River and those breeding in the Nzoia. These findings contradict the previous observations of Carlsson (1970) and Raybould and White (1979) who found in the rivers of western Kenya only one form of *S. damnosum*, the 'Nkusi' form. To resolve this discrepancy we collected *S. damnosum* s.l. larvae from the Thiba and the Nyamindi rivers and compared them to larvae collected from the Lusumu and the Nzoia rivers using isozyme characterization by cellulose acetate membrane electrophoresis (CAE).

MATERIALS AND METHODS

Study areas: The collecting sites for this study were two widely separated geographical zones, one around Mt. Kenya and the other in Nyanza and Western provinces. These localities were selected from previous *Simulium* study areas (Mebrahtu et al. 1986).

We collected from two sites in the Mt. Kenya area: the Thiba River where it crosses the Embu-Nairobi road (0°42′S and 37°21′E) and the Nyamindi River in the Mt. Kenya area (0°38′S and 37°22′E). The Thiba River has a rocky bed that is covered by mud eroding from the fertile soils of the adjoining rice fields. The Nyamindi River bed is composed of boulders and rocky material covered with algae and other small aquatic plants. We also collected from two rivers in western Kenya, the Lusumu as it runs from Mt. Elgon (0°19′N and 34°35′E) and the Nzoia (0°35′N and 34°48′E) on the Nandi Hills escarpment. Their river beds are mud, interspersed with rocks and gravel. Both rivers traverse an open riverine forest in the areas of study. Sixth and seventh stages of *S. damnosum* s.l. larvae were identified with a stereomicroscope using the keys of Freeman and de Meillon (1953), Crosskey (1969) and Mebrahtu and Mekuria (1979). The larvae were kept in labelled NUNC-tubes and frozen in liquid nitrogen immediately after collection.

Preparation of extracts, buffers, conditions for electrophoresis and data analysis: Extracts of

Simulium damnosum s.l. larvae were prepared using the method of Kreutzer et al. (1977), and Kreutzer (1979), as modified for *Simulium* (Mebrahtu et al. 1986). Briefly, the preparation of cell, reaction and membrane buffers, the staining components in noble agar as well as electrophoresis conditions were prepared using the methods of Kreutzer and Galindo (1980) and Kreutzer and Christensen (1980) with small modifications for blackflies (see Mebrahtu et al. 1986). Electrophoresis equipment (Helena Laboratories, Beaumont, TX) with an LKB BROMMA 2121 Power Supply, 300V/100 mA, (LKB-Produckter AB, Bromma, Sweden) was used.

The enzymes studied were glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase (6PGD), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), xanthine dehydrogenase (XDH), glutamate oxaloacetate (GOT), hexokinase (HK), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI).

Numerical designations of the alleles in all the tables were based on relative electromorph migrations. For each enzyme locus the most common electromorph of *S. damnosum* s.l. from the four breeding localities was designated the standard with a value of '100.' All other Rf values are relative to the standard.

The observed allele frequencies were compared to values expected if random mating were taking place (according to Hardy-Weinberg equilibrium). That is, contigency chi-square statistics based on the observed members of each allele at a single locus (e.g., PGI) were used to compare allele frequencies among the different breeding sites.

RESULTS AND DISCUSSION

The enzymes PGM, PGI, HK, ME and XDH were very active and stained rapidly with good to excellent resolution. Enzymes PGM, HK and PGI produced inter- and intrapopulation variation (Tables 1 and 2). The enzymes ME, XDH and 6PGD produced good to excellent bands but their Rf values were the same in all assays. Similarly, GOT, MDH, LDH and G6PD produced faint bands and could not be used for useful interpretation of zymograms. Both GOT and MDH showed anodal and cathodal migra-

Table 1. Number of *Simulium damnosum* s.l. from each locality either homozygous or heterozygous for various alleles at the *Pgm*, *Hk*-1,2 and *Pgi* loci.

Locus	Isozyme pattern	Allele	Lusumu	Nzoia	Thiba	Nyamindi
Pgm	1	100/113	3	2		
	2	100/113	14	13		
	3	100/113	25	27		
	4	100:100	6	8		
	5	126:126			28	33
	6	116/129			0	2
	7	126/137			7	8
	8	126/137			5	5
Hk-1	1	100:100	33	29		
	2	92/100	16	14		
	3	100/106	17	21		
	4	106/110	4	6		
	5	92:92			27	26
	6	79/92			21	25
	7	79/92			15	16
	8	77/88			10	8
Hk-2	1	54/79	33	29		
	2	54/79	16	14		
	3	54/79	17	21		
	4	79/85	4	6		
	5	48/75			27	26
	6	48/75			21	25
	7	48/75			15	16
	8	42/69			10	8
Pgi	1	100/190	4	4		
	2	100/250	10	4		
	3	100:100	93	97	30	25
	4	100/200			63	61
	5	100/300			1	1
	6	200:200			16	15

Table 2. Geographic variation of allele frequencies in *Simulium damnosum* s.l. calculated from Table 1.

Locus	Allele	Lusumu	Nzoia	Thiba	Nyamindi
Pgm	100	0.56	0.42		
	113	0.44	0.58		
	116			–	0.02
	126			0.85	0.82
	129			–	0.02
	137			0.15	0.14
Hk-1	77			0.07	0.05
	79			0.25	0.28
	88			0.07	0.05
	92	0.11	0.10	0.62	0.62
	100	0.71	0.66		
	106	0.15	0.19		
	110	0.03	0.05		
Hk-2	42			0.07	0.05
	48			0.43	0.45
	54	0.47	0.46		
	69			0.07	0.05
	75			0.43	0.45
	79	0.50	0.50		
	85	0.03	0.04		
Pgi	100	0.93	0.96	0.56	0.55
	190	0.02	0.02		
	200			0.43	0.44
	250	0.05	0.02		
	300			0.01	0.01

tions. The rest of the enzyme assays migrated toward the anode of the electrophoretic cell.

Phosphoglucomutase (PGM). In both the Mt. Kenya and western Kenya *S. damnosum* s.l., eight different isozyme patterns were identified at the PGM locus. The zymograms obtained with PGM assays from the Thiba and Nyamindi rivers on single *S. damnosum* s.l. larval specimens were distinctly different than those from the Lusumu and the Nzoia using the same fly species and electrophoretic conditions. Diagrammatic representation of the PGM enzyme pattern is given in Mebrahtu et al. (1986). The number of specimens assayed from each locality and the geographic variation of allele frequencies in *S. damnosum* s.l. at the *Pgm* locus are shown in Tables 1 and 2 respectively. This enzyme (PGM) was found to be fast staining and with a very clear resolution which easily differentiated the *S. damnosum* s.l. from the two geographical areas in Kenya. A sample zymogram for PGM is given in Fig. 1.

Tables 1 and 2 show that there was no overlap of *Pgm* alleles between the *S. damnosum* s.l. studied from the Mt. Kenya sites and the Western and Nyanza provinces. However, within these populations polymorphism at the *Pgm* locus was evident.

In Fig. 2 of Mebrahtu et al. (1986), there are often secondary diffuse areas of staining adjacent to the main bands (e.g., see pattern I, II and VIII). When assigning Rf values to the

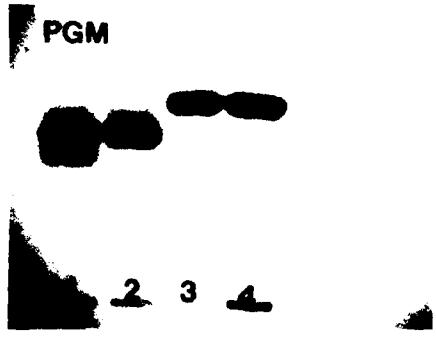

Fig. 1. Sample zymogram for PGM enzyme.

Fig. 2. Sample zymogram for HK enzyme.

PGM isozyme pattern and when scoring allele frequencies, these faint or speckled bands were disregarded. There are several possible interpretations for such bands: they may be artifacts of refrigeration storage, or the heavy bands may be nuclear and mitochondrial in origin, while the faint bands may be cytoplasmic (Dr. H. Townson, personal communication). In our study, we always observed these faint bands, whether the material was freshly collected or stored in liquid nitrogen for longer periods. Patterns I, II and III are phenotypic variations of the same genotype (loc. cit.). Hence, if we disregard the faint bands in Fig. 2 of Mebrahtu et al. (1986), there are only two alleles for patterns I–IV and three alleles for patterns V–VII. The four bands in patterns V–VIII are allelic and always different on the cellulose acetate membrane. Therefore, the possible subpopulations within the Thiba and the Nyamindi in the Mt. Kenya area seem to be greater than that of Western and Nyanza provinces.

Hexokinase (HK). Hexokinase produced eight distinct isozyme patterns from the two populations of *S. damnosum* s.l. collected from each area. The patterns, shown diagrammatically in Fig. 6 of Mebrahtu et al. (1986) showed no overlap between the two populations with HK electrophoresis. Furthermore, HK demonstrated intraspecific polymorphism within each breeding locality, as shown on Tables 1 and 2. A sample of an HK zymogram is shown in Fig. 2. However, this variation was not significant between Lusumu and Nzoia or between the Thiba and the Nyamindi. Because there appear to be at least two forms of hexokinase, presumably representing products of two separate loci, Rf values were calculated as HK-1 and HK-2. A reasonable interpretation of the HK patterns seen in the Kenyan *S. damnosum* s.l. is that the upper group of bands represent activity at one locus and the lower group activity at another one or two loci (Dr. H. Townson, personal communication). If we ignore the apparent slight difference in mobility of the two bands for pattern VIII, the patterns of the upper bands may be the expression of five alleles at a single locus. If this is the case, only one allele is shared between the populations from Mt. Kenya and those from Nyanza and Western provinces (see Tables 1 and 2).

Phosphoglucose isomerase (PGI). Diagrammatic representation of PGI electrophoretic assays is shown in Fig. 3. Pictures of sample zymograms are shown in figures 4 and 5. Unlike PGM and HK, PGI shows a clear interpopulation polymorphism in only 25% of single fly assays between the two populations of *S. damnosum* s.l., as shown in Table 1. This polymorphism of PGI was significantly different between the two populations of *S. damnosum* s.l.

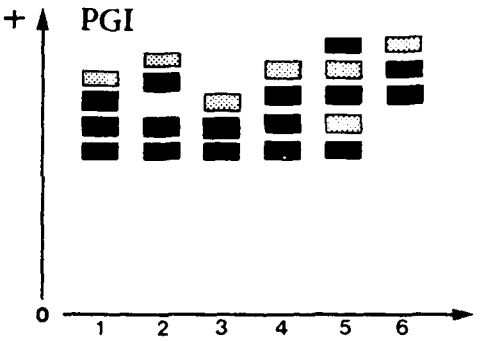

Fig. 3. Diagrammatic representation of PGI enzyme banding patterns.

Fig. 4. Sample zymogram for PGI enzyme with individual larval variation.

Fig. 5. Sample zymogram for PGI enzyme with another individual larval variation.

in Nyanza and Western provinces and those in Mt. Kenya areas. On the other hand, there was no significant population variation between the *S. damnosum* s.l. from Lusumu and Nzoia and those from Thiba and Nyamindi.

Unlike our first diagrammatic representation of PGI from *S. damnosum* s.l. (Mebrahtu et al.

1986) which had eight isozyme patterns obtained from larvae, pupae and adults, Fig. 3 shows only six different patterns from single larval assays of same flies. Therefore, our previous patterns I and VIII do not correspond to any of our present larval PGI patterns. These patterns may only be expressed by pupae and adults and not by larvae.

This study has demonstrated that three enzyme substrate systems, namely PGM, HK, and PGI, differentiate *S. damnosum* populations from two widely separated geographical areas in Kenya. Thus these three enzymes may be used to identify these two sylvatic populations of *S. damnosum* s.l. in Kenya. These results are similar to earlier studies using cytotaxonomy (Carlsson 1970) that identified only one form of *S. damnosum* s.l. from rivers in western Kenya.

Simulium damnosum s.l. from Thiba and Nyamindi rivers belong to the same population but different than those from Lusumu and Nzoia. This population in the Mt. Kenya area shows an intraspecific enzyme polymorphism. Meredith and Townson (1981) gave four different enzyme patterns of *S. damnosum* s.l. using PGM starch-gel electrophoresis assays from Tanzania collections. Their B_2, B_2C, CD and D correspond to the enzyme patterns IV, II, VI and V shown in Fig. 2 of Mebrahtu et al. (1986), respectively.

Although this may not mean that the Tanzanian forms are identical to those found in Kenya, there exists a similarity in the enzyme profiles of *S. damnosum* s.l. in East Africa.

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