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ELECTROPHORETIC ANALYSIS OF GENE-ENZYME SYSTEMS IN CHABERTIA OVINA

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Abstract. In *Chaberia ovina* species an electrophoretic study of 15 loci of the following enzymes has been conducted: glucose phosphate isomerase, mannose phosphate isomerase, glucose-6-phosphate dehydrogenase, glutamate-oxaloacetate transaminase, superoxide dismutase, isocitrate dehydrogenase, hexokinase, adenylate kinase, malate dehydrogenase, malic enzyme, carbonic anhydrase and 6-phosphogluconate dehydrogenase. The genetic variability has been relatively high, with 40 % polymorphism values noted, an 0.10 mean heterozygosity observed and an 0.17 mean heterozygosity expected. The greater part of the allele frequencies were not in Hardy-Weinberg equilibrium.

Chabertia ovina is a wide spread parasite found in the colon of goats, sheep and other ruminants. The adult worms affix to the mucus of the colon, producing haemorrhage, anaemia and even the death of the host.

The application of electrophoresis to detect specific proteins has been of great value in the analysis of gene-enzyme systems, providing an increased understanding of the process of evolution.

These electrophoretic methods permit calcuation of the allele frequencies of many loci in different populations, and in this way provide information regarding genetic variability.

In recent years, these studies have been increasing in parasitology, specially in the nematode groups (Cianchi et al. 1985). However, to date, the ascaridoids have been most studied (Zee et al. 1970, Bullini et al. 1978, Nadler 1986, Nascetti et al. 1986). We think it would be of great interest to extend these studies to other species of nematode parasites.

MATERIALS AND METHODS

548 adult worms of *C. ovina* from *Capra hircus* were collected from the slaughter-house in Granada All the specimens collected were frozen at —70 °C for later analysis.

Crude extracts of whole nematodes were crushed in 20 µl of distilled water and absorbed onto 4 × 5 mm filter paper (Whatman#3MM), which was inserted in starch gel trays (21 per tray).

Starch gels were run horizontally and were prepared using 11 % (w/v) electrostarch. Electrophoresis was conducted at 5 °C at a constant voltage of 8V/cm until the bromohpenol blue tracking dye had migrated 10 cm.

Enzymatic activity was detected in gels using standard tchniques (Nacetti et al. 1986). The following enzymes were studied: glucose phosphate isomerase (GPI), mannose phosphate isomerase (MPI), glucose-6-phosphate dehydrogenase (G6PDH), glutamate-oxaloacetate transaminase (GOT), superoxide dismutase (SOD), isocitrate dehydrogenase (IDH), hexokinase (HK), adenylate kinase (ADK), malate dehydrogenase (MDH), malic enzyme (ME), carbonic anhydrase (CA) and 6-phosphogluconate dehydrogenase (6PGDH).

Parameters of genetic variability used: Ho- Observed mean heterozygosity; He- Expected mean heterozygosity; Pr- Proportion of polymorphic loci at the 1 % criterion; A- Mean number of alleles per locus.

RESULTS

For each locus, from 20—70 specimens were tested. The allele frequencies observed and mean heterozygosity are reported in Table 1. Alleles represented by frequencies < 0.01 have been excluded. In Table 2, the expected and observed frequencies are shown for each phenotype analysed. Polymorphism for 7 out of 15 loci examined was found, with 2—4 alleles. (Mean number of alleles per locus: 1.8.)

The genetic variability is summarized in Table 3, the observed mean heterozygosity per locus was rather high. The loci G6PDH, SOD, IDH_1, IDH_2, MDH_1, MDH_2 and EM_2 were monomorphic in all the samples. Three loci (MPI, GOT and ADK) have significatively different distributions of alleles (Hardy-Weinberg equilibrium for codominant alleles) with $P < 0.01$, and one locus (CA) with $P < 0.05$. All the other loci have allele frequencies in equilibrium; see Table 2.

Table 1. Allele frequencies and %-heterozygosity at 15 loci of *Chabertia ovina*

Loci	No Specimens	Alleles	Alleles Frequencies	% Heter. Observed	% Heter. Expected
PGI	70	100	0.99	1.42	1.41
		98	0.01		
MPI	40	100	0.82	25.00	30.40
		98	0.09		
		96	0.09		
G6PDH	57	100	1.00	—	—
GOT	49	100	0.51	26.53	61.89
		98	0.22		
		95	0.27		
SOD	68	100	1.00	—	—
IDH_1	20	100	1.00	—	—
IDH_2	20	100	1.00	—	—
HK	20	100	1.00	—	—
ADK	20	100	0.53	5.00	49.87
		102	0.47		
MDH_1	21	100	1.00	—	—
MDH_2	21	100	1.00	—	—
EM_1	39	100	0.68	33.33	43.55
		98	0.32		
EM_2	40	100	1.00	—	—
CA	42	100	0.63	40.47	55.18
		102	0.10		
		98	0.18		
		95	0.09		
6PGDH	21	100	0.90	19.04	17.57
		103	0.07		
		97	0.03		

DISCUSSION

These investigations of *C. ovina* yielded some high values of genetic variabilities; an average heterozygosity of 0.10 was observed. In *Parascaris equorum*, Bullini et al. (1978) found values of 0.0088; for this same species, Nadler (1986) reported a value of 0.085. This same author also studied two other ascarids, *Toxocara canis* and *Toxocara cati*, and found a mean heterozygosity of 0.135 and 0.137, respectively.

The percentage of polymorphic loci obtained for the object species of this study

is 40 %. In other nematodes such as *T. canis* and *T. cati*, Nadler (1986) obtained values of 33.3 % and 38.9 %. According to Nadler, this considerable genetic diversity in a parasitic organism does not agree with the theory that the intestinal medium selects the monomorphic individuals among the endoparasites.

Table 2. Phenotype frequencies and x^2-test at 15 loci in *Chabertia ovina*

Loci	Phenotypes	Observed Frequencies	Expected Frequencies	X^2	P
PGI	100/100	0.99	0.99	0.0003	<0.98
	100/98	0.01	0.01		
MPI	100/100	0.70	0.68	11.901	<0.01
	100/98	0.08	0.14		
	100/96	0.17	0.14		
	98/98	0.05	0.01		
G6PDH	100/100	1.00	1.00	—	—
GOT	100/100	0.44	0.26	30.943	<0.001
	100/98	0.06	0.22		
	100/95	0.10	0.27		
	98/98	0.14	0.05		
	98/95	0.10	0.11		
	98/95	0.16	0.07		
SOD	100/100	1.00	1.00	—	—
IDH_1	100/100	1.00	1.00	—	—
IDH_2	100/100	1.00	1.00	—	—
HK	100/100	1.00	1.00	—	—
ADK	100/100	0.50	0.27	16.190	<0.001
	100/102	0.05	0.49		
	102/102	0.45	0.22		
MDH_1	100/100	1.00	1.00	—	—
MDH_2	100/100	1.00	1.00	—	—
EM_1	100/100	0.51	0.46	2.148	<0.50
	100/98	0.33	0.44		
	98/98	0.16	0.10		
EM_2	100/100	1.00	1.00	—	—
CA	100/100	0.48	0.39	15.512	<0.05
	100/102	0.10	0.12		
	100/98	0.14	0.22		
	100/95	0.07	0.12		
	102/98	0.07	0.03		
	102/95	0.02	0.01		
	98/98	0.07	0.03		
	95/95	0.05	0.01		
6PGDH	100/100	0.81	0.81	0.232	<0.90
	100/103	0.14	0.12		
	100/97	0.05	0.04		

Table 3. Genetic variability at 15 loci of *Chabertia ovina*

Population	Ho	He	Pr	A
C. ovina	0.10	0.17	40	1.8

HO — Observed mean Heterozygosity per locus
He — Expected mean Heterozygosity per locus
Pr — Proportion of polymorphic loci at the 1% criterion
A — Mean number of alleles per locus

Five out of seven polymorphic loci were not in Hardy-Weinberg equilibrium. A possible explanation for this alteration in codominant allelic heredity is that it may be caused by an existing high consanguinity in the population, which may be responsible for the appearance of rare alleles in homozygosity.

As a rule, the consaguinity may have little effect on the change of frequencies ot united genics, but it can have an important effect on the frequency of the homozygotes (Strickberger 1978).

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