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Isozymic comparison of five laboratory lines of tsetse flies belonging to the two subspecies of *Glossina palpalis* (Diptera: Glossinidae)

BY P. ELSEN* AND P. ROELANTS
Prince Leopold Institute of Tropical Medicine, 155 Nationalestraat, B-2000 Antwerp, Belgium

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Three laboratory colonies of *Glossina palpalis palpalis* and two of *G. p. gambiensis* have been characterized by means of 14 polymorphic enzyme loci. The presence/absence of some alleles for three enzymes (octanol dehydrogenase, phosphoglucomutase and aldehyde oxidase) distinguished the two subspecies. Other differences in allozymes could not be used to discriminate between subspecies but could be used to distinguish populations within each of the subspecies. The genetic differences between populations of a given subspecies are briefly discussed.

By investigating five enzymes, Elsen *et al.* (1994) demonstrated a significant genetic difference between two laboratory colonies of *Glossina palpalis gambiensis*, which also differed in terms of vectorial competence. The genetic difference observed appears to be the result of differences in the breeding conditions under which the flies were maintained (Elsen *et al.*, 1993) and genetic drift since the foundation of the lines.

In the present study, which represents an extension of these earlier investigations, the isozymatic characterization was expanded to 14 polymorphic loci, and three laboratory colonies of *G. p. palpalis* were added to the two *G. p. gambiensis* lines already studied. Establishment of the current enzymatic profile of each line should permit detection of future genetic drift, which must be taken into account when trypanosomes are transmitted by the flies under experimental conditions.

MATERIALS AND METHODS

The tsetse flies used, all from laboratory colonies, were three lines of *G. p. palpalis* [Kaduna (KA), TAN and Zaire (ZA)] and two of *G. p. gambiensis* [Maisons-Alfort (MA) and Bobo-Dioulasso (BO)].

KA has been maintained in the laboratories of the International Atomic Energy Agency in Vienna since 1974, was developed from 200 flies collected near Kaduna, Nigeria, and has been kept as a large colony of about 65 000 flies since 1987. Elsen *et al.* (1993) described the histories and maintenance of the four other lines, TAN, ZA, MA and BO, which are held in the laboratories of the Institute of Tropical Medicine (ITM) in Antwerp. TAN is a mutant of KA, from which it originated by a single mutation on the X chromosome (D'Haeseleer *et al.*, 1987); one male isolated in 1983 copulated with six wild females to found a colony now maintained at the level of 500 flies. The ZA line, maintained at about 1500 flies since 1972, derives from five flies collected from Lower Zaire in 1968 and another 153 flies collected from the same area in 1972. The MA line developed from 550 pupae produced in Burkina Faso in 1972 and has been maintained in the ITM as 1500 flies. BO was developed from MA (as a source of large numbers of male flies for tsetse control via sterile-male release) and began in Bobo-

* E-mail: pelsen@entom.itg.be; fax: +32 3 2161431.

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TABLE 1
Isozyme electrophoresis conditions

*Enzyme**	*Migration buffer*	*Application*	*Run time (min)*
MDH	Helena HR Electra buffer	Central	30
ME	(0.5 M) Tris–EDTA–borate (pH 8.0)	Cathode	18
6PGDH	(0.155 M) Tris–citrate (pH 7.0)	Cathode	24
PGM	(0.1 M) Tris–maleic–EDTA (pH 7.4)	Cathode	24
GPI	(0.5 M) Tris–EDTA–borate (pH 8.0)	Cathode	24
HK	(0.5 M) Tris–EDTA–borate (pH 8.0)	Cathode	18
ODH	(0.025 M) Tris–glycine (pH 8.5)	Cathode	30
AO	(0.155 M) Tris–citrate (pH 7.0)	Cathode	24
EST	Helena HR Electra buffer	Cathode	30
ACP	(0.015 M) Tris–EDTA–borate (pH 7.8)	Central	36
XO	(0.015 M) Tris–EDTA–borate (pH 7.8)	Cathode	42

* See Materials and Methods for explanation of abbreviations.

Dioulasso (Burkina Faso) with 300 pupae. The BO colony at the ITM began in 1987 and has been maintained at the level of 500 flies. Since their founding all the colonies have been maintained under the same conditions (25°C and 85% relative humidity) and fed on guinea pigs.

Extracts from 50 males and 50 females of each colony were subjected to electrophoresis on cellulose acetate and then stained to show the locations of 11 enzymes: acid phosphatase (EC 3.1.3.2; ACP); aldehyde oxidase (EC 1.2.3.1; AO); esterase (EC 3.1.1.1; EST); glucose-phosphate isomerase (EC 5.3.1.9; GPI); hexokinase (EC 2.7.1.1; HK); malic enzyme (EC 1.1.1.40; ME); malate dehydrogenase (EC 1.1.37; MDH); octanol dehydrogenase or alcohol dehydrogenase (EC 1.1.1.1; ODH or ADH); phosphoglucomutase (EC 2.7.5.1; PGM); 6-phosphogluconate dehydrogenase (EC 1.1.1.44; 6PGDH); and xanthine oxidase (EC 1.2.3.2; XO). The running conditions (Table 1) and staining procedures used (Table 2) were adapted from those of Dujardin and Tibayrenc (1985) and Richardson *et al.* (1986).

Mean genetic diversity was estimated as the *H* index (Nei, 1987) and mean genotype diversity as the *G* index (Ben Abderrazak, 1993); details of both indices were given by Elsen *et al.* (1994). Nei's standard genetic distances (*Ds*; Nei, 1972) were also calculated.

RESULTS

The 11 enzymes investigated showed 14 polymorphic loci. The results for three enzymes distinguished the two subspecies: *G. p. palpalis* only had the first three of the five ODH (or ADH) alleles seen in *G. p. gambiensis*, and *G. p. gambiensis* only had the second and third of the three AO alleles and of the three PGM alleles seen in *G. p. palpalis* (Table 3). Although there were similar differences between the subspecies in the alleles for ACP, the sex-specific banding patterns for this enzyme (Table 4) were difficult to understand and cast doubt on the usefulness of studies of this enzyme to distinguish the two subspecies. The ACP of *G. p. gambiensis* gave three bands (A, B and C) but heterozygotes occured in both sexes only for bands A and B whereas the ACP of *G. p. palpalis* only gave bands B and C, with no heterozygotes detected in either sex.

Although the results for the other allozymes did not discriminate between the two subspecies, they could distinguish the lines. Within *G. p. palpalis*, for example, ZA differed from

TABLE 2

Staining conditions for the isozymes, showing the staining solutions made up to 10 ml with distilled water and then mixed with an equal volume of molten, 1.2% agar

	Stain buffer			*Substrate*		*Activator*		*Coenzyme(s)†*			
*Enzyme**	*Type*	*pH*	*Volume (ml)*	*Type*	*Amount*	*Type*	*Volume (μl)*	*Type*	*Amount*	*Linking enzyme*	*Stain‡*
MDH	1 M Tris–HCl	8.0	1.2	1 M Malate pH 7.0	900 μl	—		NAD	200 μl	—	NBT (500 μl) and PMS (300 μl)
ME	1 M Tris–HCl	7.0	2.5	1 M Malate pH 7.0	600 μl	1 M $MgCl_2$	250	NADP	100 μl	—	NBT (500 μl) and PMS (300 μl)
6PGDH	1 M Tris–HCl	8.0	2.5	6–Phosphogluconic acid	10 mg	1 M $MgCl_2$	250	NADP	100 μl	—	NBT (500 μl) and PMS (300 μl)
PGM	1 M Tris–HCl	8.0	2.5	Glucose–1–phosphate	20 mg	1 M $MgCl_2$	250	NADP	100 μl	8 IU G6PDH	NBT (500 μl) and PMS (300 μl)
GPI	1 M Tris–HCl	7.0	2.5	Fructose-6-phosphate	10 mg	1 M $MgCl_2$	250	NADP	100 μl	4 IU G6PDH	NBT (500 μl) and PMS (300 μl)
HK	1 M Tris–HCl	8.0	2.5	Glucose	20 mg	1 M $MgCl_2$	250	NADP	100 μl	4IU G6PDH	NBT (500 μl) and PMS (300 μl)
								ATP	10 mg		
ODH	1 M Tris–HCl	8.0	2.0	1-Octanol	400 μl	—		NAD	200 μl	—	NBT (500 μl) and PMS (300 μl)
AO	1 M Tris–HCl	8.0	1.0	Benzaldehyde	300 μl	—		—		—	NBT (500 μl) and PMS (300 μl)
EST	0.1 M Phosphate	6.3	7.0	α-Naphthyl acetate	60 mg	—		—		—	Fast Blue BB or RR (20 mg) in distilled water (1 ml)
				β-Naphthyl acetate	12 mg						
				50% Acetone	2 ml						
ACP	0.05 M Citrate	4.0	10.0	α-Naphthyl phosphate	10 mg	1 M $MgCl_2$	250				Post-coupling Fast Garnet§
XO	1 M Tris–HCl	8.0	2.0	Hypoxanthine	10 mg						NBT (500 μl) and PMS (300 μl)

* See Materials and Methods for explanation of abbreviations.

†β-Nicotinamide adenine dinucleotide phosphate (NADP) and β-nicotinamide adenine dinucleotide (NAD) were kept as stock solutions containing 50 and 25 mg/ml, respectively.

‡Nitro Blue tetrazolium (NBT) and phenazine methosulphate (PMS) were each kept as stock solutions containing 10 mg/ml.

§Using 20 mg Fast Garnet in 10 ml 0.1 M Tris–HCl, pH 7.0.

G6PDH, Glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

TABLE 3

Allele frequencies in the five populations studied, heterozygosity (h) at each locus, mean heterozygosity (H) and mean diversity (G)

			Glossina p. palpalis			Glossina. p. gambiensis	
*Locus**	*Allele*		*Zaire*	*Kaduna*	*TAN*	*Maisons-Alfort*	*Bobo-Dioulasso*
ODH	A		0.114	0.379	0.233	0.020	0.067
	B		0.657	0.500	0.583	0.520	0.456
	C		0.229	0.121	0.183	0.367	0.411
	D		0.000	0.000	0.000	0.061	0.044
	E		0.000	0.000	0.000	0.031	0.022
		h	0.503	0.592	0.572	0.589	0.617
6PGDH	A		0.582	0.582	0.708	0.119	0.148
	B		0.311	0.279	0.292	0.563	0.535
	C		0.107	0.139	0.000	0.319	0.317
		h	0.553	0.564	0.414	0.568	0.591
ACP	A		0.033	0.000	0.000	0.413	0.654
	B		0.567	0.353	0.257	0.393	0.279
	C		0.400	0.647	0.743	0.193	0.066
		h	0.518	0.457	0.381	0.637	0.489
MDH-1	A		0.026	0.022	0.035	0.000	0.022
	B		0.961	0.907	0.866	0.943	0.978
	C		0.013	0.071	0.099	0.057	0.000
		h	0.077	0.173	0.239	0.108	0.043
MDH-3	A		0.000	0.011	0.011	0.000	0.000
	B		1.000	0.989	0.989	0.978	1.000
	C		0.000	0.000	0.000	0.022	0.000
		h	0.000	0.021	0.021	0.042	0.000
PGM	A		0.012	0.398	0.019	0.000	0.000
	B		0.938	0.283	0.957	0.833	0.903
	C		0.049	0.319	0.025	0.167	0.097
		h	0.117	0.660	0.084	0.278	0.175
GPI	A		0.000	0.019	0.038	0.023	0.156
	B		0.139	0.962	0.758	0.667	0.394
	C		0.785	0.019	0.189	0.264	0.381
	D		0.076	0.000	0.015	0.046	0.069
		h	0.359	0.074	0.389	0.483	0.670
EST-1	A		0.081	0.130	0.135	0.073	0.157
	B		0.301	0.362	0.507	0.533	0.404
	C		0.500	0.362	0.324	0.333	0.416
	D		0.118	0.145	0.034	0.060	0.024
		h	0.639	0.699	0.619	0.595	0.639
EST-2	A		0.286	0.287	0.087	0.241	0.299
	B		0.304	0.338	0.293	0.393	0.433
	C		0.339	0.287	0.598	0.152	0.134
	D		0.071	0.087	0.022	0.214	0.134
		h	0.706	0.713	0.548	0.719	0.687
AO	A		0.452	0.671	0.580	0.000	0.000
	B		0.530	0.296	0.413	0.787	0.750
	C		0.018	0.033	0.007	0.213	0.250
		h	0.515	0.461	0.493	0.335	0.383
XO	A		0.483	0.195	0.132	0.393	0.250
	B		0.325	0.271	0.245	0.536	0.508
	C		0.192	0.534	0.623	0.071	0.242
		h	0.624	0.603	0.535	0.554	0.621

TABLE 3–*continued*

		Glossina p. palpalis			Glossina. p. gambiensis	
*Locus**	*Allele*	*Zaire*	*Kaduna*	*TAN*	*Maisons-Alfort*	*Bobo-Dioulasso*
HK-1	A	0.042	0.130	0.036	0.292	0.463
	B	0.958	0.851	0.821	0.708	0.538
	C	0.000	0.019	0.143	0.000	0.000
	h	0.055	0.259	0.304	0.414	0.497
HK-2	A	0.411	0.590	0.441	0.026	0.019
	B	0.589	0.410	0.559	0.974	0.981
	h	0.484	0.484	0.493	0.051	0.038
ME	A	0.412	0.191	0.015	0.587	0.646
	B	0.426	0.515	0.559	0.370	0.292
	C	0.162	0.294	0.426	0.043	0.063
	h	0.622	0.612	0.506	0.517	0.494
	H and (S.D.)	0.182 (0.060)	0.206 (0.062)	0.184 (0.052)	0.201 (0.051)	0.230 (0.057)
	G	0.575	0.780	0.694	0.727	0.813

* See Materials and Methods for explanation of abbreviations.

KA and TAN [in the absence of allele C in HK-1, allele A in GPI and alleles A and C in MDH-3 (monomorphic for allele B in MDH-3)], KA differed from ZA and TAN (in the absence of allele D in GPI) and TAN differed from KA and ZA (in the absence of allele C in 6PGDH). Within *G. p. gambiensis*, BO only showed alleles A and B of MDH-1 (whereas MA only showed alleles B and C) and allele B of MDH-3 (whereas MA showed alleles B and C).

Nei's genetic distances, calculated between lines for the whole set of polymorphic loci observed, are given in Table 5. The *G* diversity indices, when calculated for at least seven of the 14 polymorphic loci, were almost 100% for all five lines. However, when calculated for any six of the 14 polymorphic loci, they revealed significant differences between the five lines. The values of *G* and *H* (Table 3) both indicate that ZA has the lowest diversity ($G=0.575$; $H=0.182$), followed by TAN ($G=0.694$; $H=0.184$), MA ($G=0.727$; $H=0.201$); KA ($G=0.780$; $H=0.206$) and BO ($G=0.813$; $H=0.230$). However, none of the between-line differences in *H* is significant (Student's *t*-tests; $P>0.05$ for each) and only two of those in *G* (i.e. ZA *v.* KA, and ZA *v.* BO) are significant (χ^2 and Wilcoxon's sum-of-ranks tests; $P<0.05$ for each).

DISCUSSION

In the present study, the results for three of the enzymes investigated (ODH, PGM and AO) appeared to be subspecies-specific.

Although the results for ACP might also discriminate between the subspecies, the banding patterns for this enzyme were difficult to interpret; all the tsetse flies investigated gave only one band (A, B or C) or were AB heterozygotes. Band C cannot belong to a second locus because the band would then be found in all specimens. The existence of a null allele could not explain the banding patterns seen. Perhaps heterozygotes involving the C allele have reduced fitness compared with the other flies.

None of the between-line differences in *H* and only two of those in *G* were statistically significant. However, the parallel trends in these two indices are interesting and appear generally to reflect differences in the variability of the conditions under which each line has been maintained (Elsen *et al.*, 1994), or the length of time each has been maintained under constant conditions of temperature, humidity and feeding method in the laboratory. Thus the lowest values for *G* and *H* were those for ZA (constant conditions since 1969),

TABLE 4

The distribution of the phenotypes for acid phosphatase in the five lines examined, amongst male (M) and female (F) flies

	G. p. palpalis						G. p. gambiensis			
	Zaire		*Kaduna*		*TAN*		*Maisons-Alfort*		*Bobo-Dioulasso*	
Phenotype	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
AA	—	—	—	—	—	—	12	16	19	21
BB	24	12	17	7	10	9	12	15	8	7
CC	11	13	16	28	32	25	7	7	—	4
AB	—	—	—	—	—	—	4	2	5	4
AC	—	—	—	—	—	—	—	—	—	—
BC	—	—	—	—	—	—	—	—	—	—
Any*	35	25	33	35	42	34	35	40	32	36

* Allelic interpretation was not possible for all the flies investigated.

TABLE 5
Matrix of Nei (1972) genetic distance coefficients for the five lines of tsetse investigated

Population	*Population*				
	Zaire	*Kaduna*	*TAN*	*Maisons-Alfort*	*Bobo-Dioulasso*
Glossina p. palpalis	—	0.181	0.118	0.142	0.162
Zaire		—	0.075	0.258	0.328
Kaduna			—	0.228	0.275
TAN					
Glossina p. gambiensis					
Maisons-Alfort				—	0.024
Bobo-Dioulasso					—

followed, in ascending order, by MA (constant conditions since 1972), KA (constant conditions since 1976) and BO (constant conditions since 1987). TAN (bred in constant conditions since 1983) does not fit this trend because its mean heterozygosity per locus, which lies between those of ZA and MA, is lower than expected. This is probably a consequence of the strong inbreeding of this line, which was developed from just one mutant male copulated to six wild females (D'Haeseleer *et al.*, 1987; Elsen *et al.*, 1993).

The level of genetic diversity observed does not seem to be associated with geographical origin or systematic status (MA being separated from BO by KA, and KA being separated from TAN by MA).

Nei's genetic distances (Table 5) also indicate the levels of genetic difference. They confirm clearly that breeding subpopulations (i.e. lines) isolated from the same geographical population of a given subspecies are genetically nearer to each other (i.e. MA *v.* BO, and KA *v.* TAN) than populations of the same subspecies from different geographical origins and, *a fortiori*, populations of different subspecies.

MA and BO, although from the same subspecies and the same geographical locality, did differ from each other in the allelic pattern at the two MDH loci, confirming the differences observed earlier (Elsen *et al.*, 1994). These differences are presumably the result of genetic drift while these lines have been held in laboratory colonies, as Gooding (1990) proposed for laboratory lines of *G. m. morsitans*. In the absence of comparisons of wild-caught flies, the question arises as to how far the other differences observed in the present study are due to the same phenomenon. The present results are therefore insufficient to conclude that there are geographical differences between populations of the same subspecies (although this has already been demonstrated for *G. pallidipes*; Gooding and Moloo, 1994), but do demonstrate genetic differences between different laboratory lines of the same subspecies. For research involving laboratory lines, therefore, attention has to be paid to these differences before conclusions at the specific or subspecific level can be made and extrapolated to field conditions. Genetic characterization of the lines employed is important for parasitologists because laboratory lines differ in their susceptibility to trypanosome infection (Elsen *et al.*, 1994).

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