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Preliminary data on the genetic differentiation of *Onchocerca volvulus* in Africa (Nematoda: Filarioidea)

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Summary

Data are reported on the genetic structure of three *Onchocerca volvulus* populations, respectively from Mali (savanna), Ivory Coast (forest), and Zaire (forest gallery in savanna). Electrophoretic analysis, carried out on 25 gene-enzyme systems, has shown a remarkable genetic heterogeneity existing within *O. volvulus*. Zaire and West Africa populations appear chiefly differentiated at *Mdh-1* and *6Pgdh* loci, their average Nei's genetic distance being 0.11. In West Africa Nei's *D* found between the savanna and forest samples is 0.04. The savanna population from Zaire is more similar to the savanna one from Mali ($D = 0.09$) than to the forest one from Ivory Coast ($D = 0.13$). This appears mainly due to the loci *Ldh* and *Hbdh* (possibly linked), some alleles of which seem to be selected for in forest populations (Ldh^{110}, $Hbdh^{108}$), while others in the savanna ones (Ldh^{100}, $Hbdh^{100}$). The hypothesis that the discrepant epidemiological patterns of human onchocerciasis are related to intrinsic differences in the parasite seems supported by the obtained data. The differences in allele frequencies found at the reported loci appear strong enough to allow biochemical identification of *O. volvulus* populations from different geographic regions and different habitats.

Key words: *Onchocerca volvulus;* electrophoresis; allozyme variation; biochemical identification; onchocerciasis.

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Introduction

Human onchocerciasis, one of the most widespread tropical diseases involving various degrees of ocular lesions up to complete blindness, is caused by the filarial worm *Onchocerca volvulus*. The clinical patterns of human onchocerciasis vary considerably from one geographical region to another and even between different bioclimatic zones within a single region.

The discrepant epidemiological picture of this disease has been observed in various endemic areas of tropical Africa, Latin America, and Yemen. One of the most important of these variations is seen in West Africa, where the prevalence of blindness due to onchocerciasis is much higher in the hot Sudan savanna and northern Guinea savanna than in the forest and preforest zones; the clinical pattern of onchocerciasis is characterized in savanna populations by a greater number of nodules, higher microfilarial concentration, more cases of dermatitis, more ocular parasitism, and higher prevalence of severe ocular lesions of all types (Prost et al., 1980; Duke, 1981). Considerable variations in the clinical picture of onchocerciasis have been observed also in central Africa; for instance severe skin and lymphatic system lesions, sometimes with elephantiasis, are present along the Uele river, Zaire, while large numbers of head nodules and high prevalence of blindness characterize the Sankuru focus in the Zaire forest (Duke, 1981). Phenomena such as differential longevity of the vector *Simulium damnosum* s.l. (Le Berre et al., 1964), seasonal as opposed to perennial transmission (Bertram, 1964), etc., have been considered, but they do not seem to represent the causal factors of the clinical variation of the disease. Currently, the most favoured hypothesis is that this variation is related to intrinsic differences in the parasites. At the morphological level, *O. volvulus* from different regions appear indistinguishable, both as microfilariae and as adult worms. Some histochemical differences among microfilariae from a number of regions (Liberia, Upper Volta, Guatemala, and Yemen) were found by Omar (1978), but their taxonomic significance is uncertain.

In order to understand the genetic differentiation of the hypothesized forms of *O. volvulus*, we started to carry out multilocus electrophoretic analysis on populations of this species from different regions. This approach, which has given important contributions to the detection of sibling species in many groups (Ferguson, 1980; Bullini, 1983), has been successfully applied by Flockhart (1982) to characterize three species of cattle parasites *Onchocerca gutturosa*, *O. lienalis* and *O. gibsoni*.

In the present paper data are reported on the genetic structure of three *O. volvulus* populations from Mali (savanna), Ivory Coast (forest), and Zaire (forest gallery in savanna).

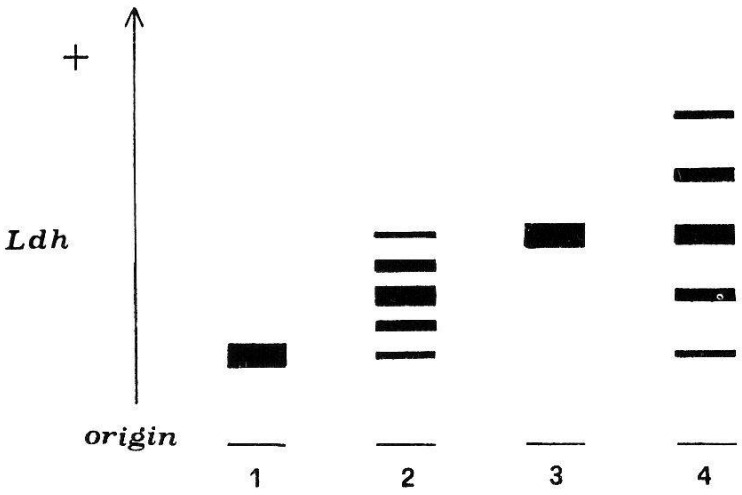

Fig. 1. Example of zymograms of lactate dehydrogenase (*Ldh*) in *Onchocerca volvulus* from Africa. 1 = Ldh^{100}/Ldh^{100}; 2 = Ldh^{100}/Ldh^{110}; 3 = Ldh^{110}/Ldh^{110}; 4 = Ldh^{100}/Ldh^{120}.

Materials and Methods

The samples analyzed consisted in adult worms and were collected from the following locations:

- Missira village, Baule river (Mali), Sudan savanna: 40 specimens;
- Trookolimplleu village, Cavally river (Ivory Coast), forest: 20 specimens;
- Mavunda village, Haute Inzia river (Zaire), forest gallery in savanna: 15 specimens.

All the specimens collected were electrophoretically analyzed. The worms (males and females) were either extracted from nodules and cleaned from human tissue prior to deep freezing in liquid nitrogen (samples from West Africa), or freezed in dry ice within their nodules (samples from Zaire). In the latter case worms were extracted from nodules immediately before electrophoresis and portions of human tissues were used as controls.

Crude homogenates were obtained from single individuals crushed in 0.1 ml of distilled water; they were absorbed in 3 by 5 mm pieces of chromatography paper (Whatman 3MM), which were inserted in starch gel trays (22 individuals per tray). Standard horizontal electrophoresis was carried out at 7–8 V/cm for 3½/4 h at 5° C. Gels were then sliced in two parts and each part stained for a specific enzyme. The following enzymes were studied: alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), hydroxybutyrate dehydrogenase (HBDH), malate dehydrogenase (MDH), malic enzyme (ME), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), glucose-6-phosphate dehydrogenase (G6PDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), superoxide dismutase (SOD), glutamate-oxaloacetate transaminase (GOT), hexokinase (HK), creatine kinase (CK), adenylate kinase (ADK), phosphoglucomutase (PGM), alkaline phosphatase (APH), acid phosphatase (ACPH), aldolase (ALD), carbonic anhydrase (CA), triose phosphate isomerase (TPI), mannose phosphate isomerase (MPI), and glucose phosphate isomerase (GPI). The staining techniques were, with some modifications, those described by Selander et al., 1971 (ADH, MDH, ME, SOD, GOT, GPI), Brewer and Sing, 1970 (LDH, PGM), Shaw and Prasad, 1970 (HBDH, IDH, 6PGDH, G6PDH, CK), Ayala et al., 1972 (G3PDH, HK, ADK, APH, ALD, TPI), and Harris and Hopkinson, 1976 (ACPH, CA, MPI). Details on the electrophoretic techniques used are given in Tables 1 and 2.

The following loci and allele designations were adopted: isozymes were numbered in order of decreasing mobility from the most anodal; the commonest allele in the reference population (Mali, savanna) was designated *100*, while the other alleles were named numerically according to their mobility relative to the *100* allele.

Table 1. Buffer systems (analytical grade reagents per litre; pH at room temperature)

Buffer system	Electrodes	Gel
1. Discontinuous Tris/citrate (Poulik, 1957)	0.3 M sodium borate, pH 8.2 (18.55 g boric acid, 2.40 g NaOH)	0.076 M Tris/0.005 M citric acid, pH 8.7 (9.21 g Tris, 1.05 g monohydrate citric acid)
2. Continuous Tris/citrate (Selander et al., 1971)	0.687 m Tris/0.157 M citric acid, pH 8 (83.2 g Tris, 30 g monohydrate citric acid)	0.023 M Tris/0.005 M citric acid, pH 8 (2.77 g Tris, 1.10 g monohydrate citric acid)
3. Tris/versene/ borate (Brewer and Sing, 1970)	0.21 M Tris/0.15 M boric acid/ 0.006 M EDTA, pH 8 (25.4 g Tris, 9.27 g boric acid, 2.20 g EDTA)	0.021 M Tris/0.02 M boric acid/ 0.0007 M EDTA, pH 8.6 (2.5 g Tris 1.24 g boric acid, 0.25 g EDTA)
4. Phosphate/citrate (Harris, 1966)	0.15 M tri-sodium citrate/ 0.24 M sodium dihydrogen phosphate, pH 6.3 (44.11 g sodium citrate, 33.12 g NaH_2PO_4)	electrode buffer diluted 1:40, adjusted to pH 6.3 with 0.2 M citric acid
5. Tris/maleate (modified from Brewer and Sing, 1970)	0.1 M Tris/0.1 M maleic acid/ 0.01 M EDTA/0.015 M $MgCl_2$/ 0.125 M NaOH, pH 7.2 (12.11 g Tris, 11.61 g maleic acid, 3.72 g EDTA, 3.05 g $MgCl_2$, 5 g NaOH)	electrode buffer diluted 1:10, pH 7.4

The following 25 loci were analyzed: *Adh*, *Ldh*, *Hbdh*, *Mdh-1*, *Mdh-2*, *Me*, *Idh-1*, *Idh-2*, *6Pgdh*, *G6pdh*, *G3pdh*, *Sod-1*, *Sod-2*, *Got-1*, *Hk-2*, *Ck*, *Adk*, *Pgm*, *Aph*, *Acph*, *Ald*, *Ca*, *Tpi*, *Mpi*, and *Gpi*. For each locus from 15 to 35 specimens per population were tested.

The genetic variability of populations was estimated with the following parameters: expected mean heterozygosity per locus (*He*), proportion of polymorphic loci at the 1% criterion (*P*), and mean number of alleles per locus (*A*). Alleles with frequencies less than 0.01 were not considered for computations. Genetic differentiation between populations was estimated using the indices of standard genetic identity (*I*) and standard genetic distance (*D*) proposed by Nei (1972). G test (Sokal and Rohlf, 1969) was used to test the statistical significance of allele frequency differences observed at single loci in the populations studied.

Results and Discussion

The allele frequencies observed in the populations tested are reported in Table 3; the data on genetic variability are summarized in Table 4. Eight out of 25 the loci analyzed appear electrophoretically monomorphic: *Mdh-2*, *G3pdh*, *Sod-1*, *Sod-2*, *Adk*, *Ck*, *Ca*, *Tpi;* the remaining 17 are polymorphic with 2–4 alleles. The level of genetic variability in *O. volvulus* is therefore rather high. The loci *Idh-2*, *Pgm*, *Aph*, *Acph*, *Ald*, *Mpi*, and *Gpi* have similar allele frequencies in the populations tested. As shown in Table 3, the Zaire population mainly differs from the West African populations at the loci *Mdh-1*, *6Pgdh*, and *Got-1*. At *Mdh-1*, *95* is the commonest allele in the Zaire sample (0.81), while its fre-

Table 2. Electrophoretic procedures

Enzyme	Buffer system	V/cm	Time	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
EC 1.1.1.1 ADH	4	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg		ethanol 3 ml propanol 3 ml		MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.27 LDH	1 or 5	8	4 h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg		L (+) lactic acid 50 mg		MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.30 HBDH	3 or 5	8	4 h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg		hydroxybutyrate 300 mg		MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.37 MDH	4	8	4 h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg		L-malic acid 1 M pH 7, 5 ml		MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.40 ME	2	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg		L-malic acid 1 M pH 7, 5 ml	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.42 IDH	2	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg		DL-isocitrate 30 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.43 6PGDH	4	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg		gluconate-6-phosphate 20 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 1.1.1.49 G6PDH	3	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg		glucose-6-phosphate 100 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 1.2.1.12 G3PDH	2	8	4 h	0.05 M Tris/HCl ph 8, 50 ml	NAD 30 mg	EC 4.1.2.13 ALD 1 mg	fructose-1,6-diphosphate 125 mg; incubate with ALD for 30′	sodium arsenate 150 mg	MTT 10 mg PMS 2 mg

Table 2 (continued)

Enzyme	Buffer system	V/cm	Time	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
EC 1.15.1.1 SOD	3 or 5	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg				MTT 10 mg PMS 2 mg agar 0.8%
EC 2.6.1.1 GOT	2	7	3½ h	0.2 M Tris/HCl pH 8, 50 ml			aspartic acid 200 mg, α-chetoglutaric acid 100 mg; adjust to pH 7.5 with 1 M Tris. Pour on gel, incubate for ½ h, then add Fast Blue BB	pyrydossal-5'-phosphate 10 mg	Fast Blue BB 150 mg
EC 2.7.1.1 HK	2	7	3½	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	ATP 10 mg glucose 45 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 2.7.3.2 CK	2	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 2.7.1.1 HK 0.2 mg	ADP 20 mg; creatine phosphate 180 mg; glucose 250 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 2.7.4.3 ADK	2	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 2.7.1.1 HK 0.2 mg	ADP 10 mg glucose 45 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 2.7.5.1 PGM	5	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	glucose-1-phosphate 80 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 3.1.3.1 APH	1	8	3½ h	0.05 M Tris/HCl pH 9, 30 ml			α-naphthyl-phosphate 100 mg	polyvinyl-pyrrolidone 150 mg $MgCl_2$ 18 mg $MnCl_2$ 18 mg NaCl 600 mg	Fast Blue BB 30 mg agar 0.8%

Table 2 (continued)

Enzyme	Buffer system	V/cm	Time	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
EC 3.1.3.2 ACPH	4	8	3½ h	0.05 M citrate pH 4.5, 50 ml			α-naphthyl-phosphate 100 mg		Fast Garnett GBC 100 mg
EC 4.1.2.13 ALD	5	8	4 h	0.05 M Tris/HCl pH 8, 30 ml	NAD 15 mg	EC 1.2.1.12 G3PDH 0.2 mg	fructose-1.6-diphosphate 125 mg	Na arsenate 125 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 4.2.1.1 CA	3	7	3½ h	0.1 M Na_2HPO_4/ 0.1 M KH_2PO_4 pH 6.5, 20 ml			4-methyl-umbelliferyl acetate 10 mg in 1 ml acetone		UV, filter paper overlay
EC 5.3.1.1 TPI	2	7	3½ h	0.05 M Tris/HCl pH 8, 25 ml	NAD 50 mg	EC 1.1.1.27 LDH 0.1 mg EC 1.1.1.8 α-GPDH 0.04 mg EC 1.2.1.12 G3PDH 0.2 mg	α-glycerophosphate 300 mg; incubate with LDH, α-GPDH, Na pyruvate × 2 h. Bring to pH 2 with 1N HCl, then to pH 8 with 1 M Tris. Add water to 50 ml and remaining reagents	Na pyruvate 300 mg Na arsenate 125 mg	MTT 10 mg PMS 2 mg
EC 5.3.1.8 MPI	3	7	3½ h	0.05 M Tris/HCl ph 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 5.3.1.9 GPI 0.04 mg	mannose-6-phosphate 25 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EC 5.3.1.9 GPI	4	7	3½ h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	fructose-6-phosphate 10 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%

Table 3. Allele frequencies observed at 25 gene-enzyme systems in African populations of *Onchocerca volvulus*. The loci *Adh*, *Me*, *Idh-1*, *G6pdh*, *G3pdh*, and *Sod-1* were not tested in the Zaire sample.

Loci	Alleles	Mali	Ivory Coast	Zaire	Loci	Alleles	Mali	Ivory Coast	Zaire
Adh	*100*	0.79	0.50		*Got-1*	*90*	0.05	–	–
	107	0.21	0.50			*95*	0.34	0.18	–
						100	0.61	0.82	0.79
Ldh	*100*	0.74	0.25	0.61		*105*	–	–	0.21
	110	0.26	0.72	0.39					
	120	–	0.03	–	*Hk-2*	*100*	0.91	0.67	1.00
						105	0.09	0.33	–
Hdbh	*100*	0.71	0.25	0.83					
	108	0.29	0.75	0.17	*Ck*	*100*	1.00	1.00	1.00
Mdh-1	*95*	0.09	–	0.81	*Adk*	*100*	1.00	1.00	1.00
	100	0.91	1.00	0.19					
					Pgm	*96*	0.01	0.08	–
Mdh-2	*100*	1.00	1.00	1.00		*100*	0.99	0.92	1.00
Me	*80*	0.02	0.04		*Aph*	*95*	0.03	–	–
	90	0.16	0.18			*100*	0.97	1.00	1.00
	95	0.31	0.32						
	100	0.51	0.46		*Acph*	*90*	0.02	–	0.04
						95	0.34	0.50	0.31
Idh-1	*100*	0.87	1.00			*100*	0.64	0.50	0.65
	104	0.13	–						
					Ald	*93*	–	–	0.06
Idh-2	*100*	0.98	1.00	1.00		*100*	1.00	1.00	0.88
	103	0.02	–	–		*107*	–	–	0.06
6Pgdh	*100*	1.00	0.93	0.35	*Ca*	*100*	1.00	1.00	1.00
	107	–	0.07	0.65					
					Tpi	*100*	1.00	1.00	1.00
G6pdh	*97*	0.11	–						
	100	0.61	0.30		*Mpi*	*100*	0.82	0.86	1.00
	105	0.28	0.70			*105*	0.18	0.14	–
G3pdh	*100*	1.00	1.00		*Gpi*	*100*	0.97	1.00	0.92
						108	0.03	–	0.08
Sod-1	*100*	1.00	1.00						
Sod-2	*100*	1.00	1.00						

Table 4. Genetic variability of three populations of *Onchocerca volvulus*, on the basis of 19 and 25 (in brackets) gene-enzyme systems

Populations	H_e	P	A
Mali (savanna)	0.16 (0.17)	0.69 (0.60)	1.8 (1.8)
Ivory Coast (forest)	0.20 (0.19)	0.50 (0.44)	1.6 (1.6)
Zaire (savanna)	0.14	0.50	1.6

H_e = expected mean heterozygosity per locus; P = proportion of polymorphic loci, at the 1% criterion; A = mean number of alleles per locus

quency does not reach 0.10 in the West African ones (complementary frequencies are found for the *100* allele). Similarly, at the *6Pgdh* locus the *107* allele is the commonest in Zaire, while it ranges from 0 to 0.07 in the West African samples (complementary frequencies are found for the *100* allele). Less differentiated is *Got-1*, the *100* allele being the commonest in all the three populations; the alleles *90* and *95*, found in West Africa, are apparently absent in the Zaire sample, while the allele *105* has only been found in Zaire. The differentiation between West African savanna and forest samples is mainly due to the loci *Ldh*, *Hbdh*, and *G6pdh*, the first two showing linkage disequilibrium, with a significant coupling of Ldh^{100}-$Hbdh^{100}$ and Ldh^{110}-$Hbdh^{108}$. Ldh^{100} and $Hbdh^{100}$ alleles are the commonest in the savanna sample (frequency >0.70), while Ldh^{110} and $Hbdh^{108}$ are the most frequent in the forest sample (frequency >0.70); the allele Ldh^{120} has been found only in the latter population. At the *G6pdh* locus, the prevalent alleles are *100* in the savanna and *105* in the forest.

The values of genetic identity and distance for the three populations tested are reported in Table 5 (the computations have been made on the 19 loci tested in all the three samples). The average genetic differentiation between Zaire and West Africa samples is 0.11, while between West African savanna and forest samples $D = 0.04$ (the same value is obtained when utilizing all the 25 loci for computation). Interestingly enough, the savanna population from Zaire is more similar to the savanna one from Mali ($D = 0.09$) than to the forest one from Ivory Coast ($D = 0.13$). This appears mainly due to the loci *Ldh* and *Hbdh*, some alleles of which seem to be selected for in forest populations (Ldh^{110}, $Hbdh^{108}$), while others in the savanna ones (Ldh^{100}, $Hbdh^{100}$). The observed values of genetic distance would indicate a rather recent differentiation between West African savanna and forest populations, while differentiation is older between Zaire and West African *O. volvulus*.

Although based on a very limited number of populations, the reported data clearly indicate a remarkable heterogeneity within *O. volvulus*. This agrees with the hypothesis that the discrepant epidemiological patterns of human onchocerciasis are related to intrinsic differences in the parasites. The electro-

Table 5. Values of Nei's standard genetic identity (above the diagonal) and distance (below the diagonal) between populations of *Onchocerca volvulus* form Mali, Ivory Coast, and Zaire, on the basis of 19 enzyme loci

	Mali (savanna)	Ivory Coast (forest)	Zaire (savanna)
Mali (savanna)	–	0.96	0.92
Ivory Coast (forest)	0.04	–	0.87
Zaire (savanna)	0.09	0.13	–

phoretic identification of *O. volvulus* populations from different regions (such as Zaire and West Africa) and from different habitats (savanna, forest) appears to be possible on the basis of their allele frequencies at a number of loci, in spite of the fact that not all the individuals can be surely identified due to the lack of fully diagnostic loci. The genetic characterization of further *O. volvulus* strains would be certainly relevant for a better knowledge of human onchocerciasis epidemiology.

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