Enzyme Polymorphisms in the *Anopheles gambiae* (Diptera: Culicidae) Complex Related to Feeding and Resting Behavior in the Imbo Valley, Burundi

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ABSTRACT A cellulose acetate electrophoresis system was used to study the *Anopheles gambiae* complex at 2 villages in Central Imbo Valley and at 1 village in South Imbo Valley (Burundi). In South Imbo, only *An. gambiae* Giles sensu stricto was present. In the drier Central Imbo, the dominant species was *An. arabiensis* Patton (97.5%); *An. gambiae* s.s. represented only 2.5%. Both species were separated readily by *Odh* and *Mpi,* because they did not share alleles at those loci. Indoor resting *An. gambiae* s.s. from South Imbo differed significantly from outdoor resting females at 2 loci, *Mpi,* and *Got-2.* In Central Imbo, total *An. arabiensis* did not differ between adjacent villages. Endophagic *An. arabiensis* significantly differed at loci *α-Gpd,* and *Idh-1* between the 2 villages, whereas no difference was observed between exophagic females. Allelic and genotypic frequencies at the locus *Mdh-2* were significantly different between indoor and outdoor biting *An. arabiensis.* Active choice for the best place to bite or to rest seemed to be associated with specific genotypes. The Nei genetic distance values were typical of conspecific populations, ranging from 0.154 to 0.160 between *An. gambiae* s.s. and *An. arabiensis.* Even though vector populations were structured at spatial and behavioral levels, they were panmictic, and thus selection of exophilic or exophagic vectors, or both, by insecticide pressure is not likely to occur.

KEY WORDS *Anopheles gambiae* complex, enzyme polymorphisms, behavior, Burundi

UNDERSTANDING OF THE behavioral heterogeneity in vector populations is crucial for developing control strategies. Selective control measures may exert a selective pressure on particular subpopulations having the same behavior. For example, indoor sprays with residual insecticide will kill the endophilic portion of a vector population, whereas the exophilic portion will not be affected, unless the 2 portions belong to the same panmictic population (Molineaux et al. 1979). Within the *Anopheles gambiae* complex, behavioral diversity is associated with intraspecific genetic polymorphism (Coluzzi et al. 1979) as well as with environmental factors such as the availability of hosts and resting places (Coosemans et al. 1992). Knowledge of population genetic structure is needed because the existence of genetically characterized subpopulations of vector species, which exhibit relevant differences in behavior could negatively affect precise evaluation of vector potential and possibilities for malaria control (Petrarca and Beier 1992).

Recognition of sibling species is important if one behavior differs. For example, *An. arabiensis* Patton shows a greater tendency to feed outdoor on cattle than *An. gambiae* Giles s.s. (Coosemans et al. 1989). In Burundi, we also observed that *An. gambiae* s.s. usually rests indoors, whereas the more abundant *An. arabiensis* leaves human dwellings after blood feeding (Smits et al. 1995).

Most studies at the intraspecific level have dealt with ecotypic diversity (Coluzzi 1992). However, few have reported behavioral differences (Hii et al. 1991; Petrarca and Beier 1992; Mnzava et al. 1994, 1995). The most common method to study intraspecific genetic polymorphism in the *An. gambiae* complex has been the analysis of paracentric floating inversions in polytene chromosomes.

We report here on an electrophoretic study of intraspecific variation in relation to the behavior of *An. gambiae* s.s. and *An. arabiensis* in the Imbo Valley in Burundi.

Materials and Method

Study Area and Mosquito Collections. The Imbo, a lowland area in the northern part of the Tanganyika Graben, is divided into South, Central, and North Imbo. Mosquitoes were collected every 14 d from March to October 1993 in Mulira (V1) and Murengeza (V2), 2 villages in Central Imbo that were described previously (Coosemans et al. 1989; Smits et al. 1995). In both villages, villagers always sleep indoors. During the night, cattle are kept in corrals located behind the houses. Five inhabited fixed stations were chosen in each village for night-biting collections (NBC), with 5 human-

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Table 1. Running conditions for cellulose acetate electrophoresis of the *An. gambiae* complex

Enzyme	Name	E.C. No.	No. loci	Quarternary structure	Buffer (soaking)	Application no. and position	Time, min
ODH	Octanol Dehydrogenase	1.1.1.73	1	Di	TG (1 + 0)	4 Cathodal	36
MPI	Mannose-phosphate Isomerase	5.3.1.8	1	Mono	A (1 + 3.5)	2 Cathodal	18
PGM	Phosphoglucomutase	2.7.5.1	2	Mono	B (1 + 14)	1 Cathodal	18
EST	Esterases	3.1.1.1	3	Mono	B (1 + 14)	3 Cathodal	24
SOD	Superoxide Dismutase	1.15.1.1	2	Di	TBS (1 + 0)	3 Cathodal	24[a]
6-PGD	6-phosphogluconate Dehydrogenase	1.1.1.44	1	Di	A (1 + 3.5)	1 Cathodal	24
α-GPD	Glycerol-3-phosphate Dehydrogenase	1.1.1.8	2	Di	A (1 + 3.5)	1 Cathodal	18
IDH	Isocitrate Dehydrogenase	1.1.1.42	2	Di	A (1 + 3.5)	1 Cathodal	18
MDH	Malate Dehydrogenase	1.1.1.37	2	Di	HR (1 + 0)	1 Central	18
GOT	Aspartate Aminotransferase	2.6.1.1	2	Di	C (1 + 1)	2 Cathodal	36
HK	Hexokinase	2.7.1.1	4		B (1 + 14)	1 Cathodal	18

Voltage, 200 V for 94 by 76 mm plate. Soaking, dilution of the running buffer to soak the gel before the sample application. Buffer, TG, 0.025 *M* Tris-glycine, pH 8.5 (Richardson et al. 1986); A, 0.155 *M* Tris, 0.043 *M* citric acid, pH 7.0; B, 0.1 *M* Tris, 0.1 *M* maleic, 0.001 *M* EDTA, pH 7.4; C, 0.5 *M* Tris-versene-borate, pH 8.0 (Shaw Prasad 1970); HR, Electra HR buffer (Helena Laboratories); TBS, 0.1 *M* Tris, 0.3 *M* boric acid sucrose 10 g/liter, pH 7.6.

[a] Voltage, 300 V.

nights indoors (NBC IN) and 5 human-nights outdoors (NBC OUT). The outdoor collectors were placed at 2 m from the house. Two collectors were in charge of each human-night collection; the 1st from 1800 to 2400 hours and the 2nd from 2400 to 0600 hours. Collectors were assigned by a rotation process to the 1st or 2nd half of the night and to the various stations. The use of night-biting collections was considered ethically acceptable because the collectors were inhabitants of the villages and remained under medical supervision. Night catches were followed in the morning by pyrethrum spray collections (PSC) in 5 inhabited houses adjacent to the fixed stations. Indoor spraying with malathion (2 g [AI]/m^2 wettable powder (WP) Luxan, The Netherlands) was started in 1985 in V1 and in 1989 in V2. One annual spray round is conducted at the beginning of April, as recommended by Coosemans and Barutwanayo (1989). In 1993, both villages were treated with lambdacyhalothrin (0.030 g [AI]/m^2 WP Zeneca, United Kingdom) on 26 April (Smits et al. 1995).

Two PSC and exit-trap collections (ETC) were organized in the middle of May in 2 fixed huts of Nyabigina (V3), a village of South Imbo. Exit traps (35 by 35 by 35 cm) (WHO 1975) were installed before sunset at the windows (3 traps per house) and emptied after sunrise just before pyrethrum spray collections. Mosquitoes could enter the house by the open eaves. Only fed mosquitoes were considered from the exit-trap collections, half gravids and gravids were disgarded from the sample.

Behavior of female mosquitoes are defined in this study as follows: mosquitoes caught by night-biting collections human-nights indoors constitute the endophagic sample, by night-biting collections human-nights outdoors the exophagic sample, by pyrethrum spray collections the endophilic sample, by exit-trap collections (only fed mosquitoes) the exophilic sample.

Mosquito Examination. Night biting mosquitoes were brought alive to the laboratory the morning after collection for morphological identification. *An. gambiae* s.l. from 1 of the 5 houses were dropped directly into liquid nitrogen, whereas those from the 4 other houses were used to study transmission dynamics (Smits et al. 1995). *An. gambiae* s.l. from the morning catches in South Imbo were identified and stored in liquid nitrogen.

Enzyme electrophoresis were carried out on cellulose acetate gels (Titan III, Helena Laboratories, United Kingdom). Head and thorax of *An. gambiae* s.l. females were homogenized in 10 μl distilled water. Eleven enzyme systems were tested. Octanol dehydrogenase (ODH) was tested on concentrated samples (10 μl per head and thorax) because of its low activity. The 10 remaining enzyme systems were tested on samples diluted with 8 μl of enzyme stabilization liquid (dithiothreitol 2 mM; aminocaproic acid 2 mM and EDTA 2 mM). Running time and buffers as well as the number of applications are shown in Table 1. Staining methods were adapted from Richardson et al. (1986) except for superoxide dismutase (SOD) (staining

system: 10 ml Tris-HCl 0.1 M pH 7.5, riboflavine 0.32 mg, EDTA 7.5 mg, p-iodonitrotetrazolium violet 2.5 mg).

Two specimens from an inbred colony of *An. gambiae* s.s. (16 cSS from the London School of Hygiene and Tropical Medicine), which is homozygous for the allele Odh^{100} and Sod^{100}, were run on each gel as a standard for ODH and SOD. For the other enzymes, 2 individuals from samples already processed were used as reference. Loci of highest mobility in the zymogram were numbered 1. The most common electromorph in *An. arabiensis* populations was designated arbitrarily as the 100 reference allele for each locus (except *Odh* and *Sod*). Mobilities for the other alleles were determined relative to this common electromorph.

Data Analysis. Samples of *An. arabiensis* and *An. gambiae* s.s. were first tested per village (NBC IN + NBC OUT + PSC in Central Imbo or PSC + ETC in South Imbo) and then split into groups corresponding to their biting and resting behavior (endophagic, exophagic, endophilic, exophilic) in each village.

A test for independence of loci was performed with a chi-square homogeneity test (Richardson et al. 1986) on the total *An. arabiensis* population. Genotypes of individuals were entered directly into the Biosys-1 software (Swofford and Selander 1981) to calculate the following 5 parameters: (1) allele frequencies; (2) heterogeneity among samples using the Pearson chi-square test for M × N contingency table with $(M - 1)(N - 1)$ degrees of freedom, where M is the number of samples and N the number of alleles. Yates correction was applied (for 2 × 2 contingency tables) when 20% or more of the expected values were <5; (3) Wright *F* statistics measure the total deviation of genotypic proportions from Hardy–Weinberg expectations. Values of the fixation indices (*F*) of the total population (*FIT*) and of subpopulations (*FIS*) vary between −1 and +1. When $F = 0$, populations are in Hardy–Weinberg equilibrium. If $F > 0$, populations show a deficit of heterozygotes. When $F < 0$, the populations show an excess of heterozygotes. The *FST* value, which varies between 0 and 1 measures the degree of genetic differentiation between subpopulations. When subpopulations are in Hardy–Weinberg equilibrium, $FIS = 0$ and $FST = FIT$. The *F* statistics values were tested for difference from 0: *FST* with $\chi^2 = 2N\,FST\,(k - 1)$; df $= (k - 1)(s - 1)$; *N*, number of individuals; *s*, number of subpopulations; *k*, number of alleles (Workman and Niswander 1970), whereas *FIS* and *FIT* with: $\chi^2 = N\,FIS^2$ or $N\,FIT^2$; df $= (k - 1)\,(s - 1)$ for *FIS* and $(k - 1)$ for *FIT* (Nei 1977); (4) exact significance probability test (analogous to Fisher exact test for 2 × 2 contingency tables) for small samples to test the hypothesis of Hardy–Weinberg equilibrium; (5) Nei's genetic distance (D) (Nei 1978), which expresses the genetic differences between 2 populations as a single number. The biological meaning of D as seen by Nei is an estimate of the number of DNA base differences per locus between populations.

Results

Species Identification. When the female populations of *An. gambiae* s.l. from Central and South Imbo were tested together for *F* statistics, *FIT* was highly significant for *Odh* ($FIT = 0.946$, $P < 0.0001$), *Mpi* ($FIT = 0.734$, $P < 0.0001$), and *Mdh-2* ($FIT = 0.197$, $P < 0.0001$). The lack of heterozygotes involving the diagnostic alleles at the *Odh* and *Mpi* loci allowed the subdivision of our samples into *An. gambiae* s.s. and *An. arabiensis,* using the electrophoretical keys of Miles (1979) and Bullini (1984). *FIS* was not significant for those 2 loci when the species were separated (*An. arabiensis*: *Odh FIS* = 0.107, $P = 0.052$; *Mpi FIS* = 0.041, $P = 0.64$. *An. gambiae* s.s.: *Odh* only 1 allele present; *Mpi FIS* = 0.006, $P = 0.99$).

In Central Imbo night-biting collections samples before house spraying comprised 2.5% (10/367) *An. gambiae* s.s. and 97.5% *An. arabiensis,* whereas 38% (20/53) of the PSC collection were *An. gambiae* s.s. In south Imbo, *An. gambiae* s.s. represented 100% (191/191) of both the endophilic and exophilic samples.

Genetic Variability. The 11 enzyme systems appeared to be encoded by 20 loci, but only 17 for *An. arabiensis* and 16 for *An. gambiae* s.s. were taken into account; *Est-1, Est-2,* and *Sod-2* showed poor resolution; *Est-3* was not legible in *An. gambiae* s.s. In the total *An. arabiensis* population, 11 loci were polymorphic (*Odh, Mpi, Pgm, Est-3 Sod-1, 6-Pgd,* α-Gpd, Idh-1, Mdh-2, Got-1, and *Got-2*), and 6 were monomorphic (*Hk-1* to 4, *Idh-2,* and *Mdh-1*). *A. gambiae* s.s. population showed 8 polymorphic loci (*Mpi, Pgm, 6-Pgd, α-Gpd, Idh-1, Mdh-2, Got-1, and Got-2*) and 8 monomorphic loci (*Odh, Sod-1, Hk-1* to *4, Idh-2,* and *Mdh-1*). Allele frequencies at polymorphic loci are summarized in Table 2, along with the *FIS* showing, when significant, samples with departure from Hardy–Weinberg equilibrium. Pyrethrum used in PSC collections has no effect on the isoenzyme patterns.

Description of the Electrophoretic Profiles. *Octanol dehydrogenase.* Four alleles at 1 *Odh* locus appeared to be species-specific. *An. gambiae* s.s. was homozygous for Odh^{100}, whereas *An. arabiensis* showed alleles Odh^{95}, Odh^{80}, and Odh^{65}.

Mannose-phosphate Isomerase. An. arabiensis was either homozygous for Mpi^{100} and Mpi^{105} or heterozygous for $Mpi^{100/105}$ and $Mpi^{100/90}$. *An. gambiae* s.s. was homozygous or heterozygous for the alleles Mpi^{130}, Mpi^{120} and Mpi^{115}.

Phosphoglucomutase. Both species showed 2 alleles Pgm^{100} and Pgm^{110}, whereas *An. gambiae* s.s. showed a 3rd allele, Pgm^{90}.

Esterase. Est-1 and *Est-2* had a very low activity and alleles were not well separated; only *Est-3* alleles were well separated. However, it was not possible to score this locus in *An. gambiae* s.s. popu-

Table 2. Allele frequencies at 11 polymorphic loci and Fis values for deviation from Hardy–Weinberg equilibrium

Locus	*An. arabiensis*						*An. gambiae* s.s.	
	V1			V2			V3	
	Endophagic	Exophagic	Endophilic	Endophagic	Exophagic	Endophilic	Exophilic	Endophilic
Odh								
N	113	178	6	74	117	28	50	132
100	—	—	—	—	—	—	1.000	1.000
95	.009	.037	—	.007	.038	.036	—	—
80	.991	.947	1.000	.973	.932	.946	—	—
65	—	.016	—	.020	.030	.018	—	—
Fis	−0.009	0.066		−0.022	0.209	−0.043		
Mpi								
N	113	186	6	76	117	29	50	125
130	—	—	—	—	—	—	.090	.028
120	—	—	—	—	—	—	.880	.968
115	—	—	—	—	—	—	.030	.004
105	.013	.024	—	.039	.026	.017	—	—
100	.978	.965	1.000	.961	.974	.983	—	—
90	.009	.011	—	—	—	—	—	—
Fis	−0.017	−0.028		−0.041	0.302**	−0.018	−0.108	0.228*
Pgm								
N	113	186	6	76	120	28	53	138
110	—	.003	—	—	.004	—	.010	.014
100	1.000	.997	1.000	1.000	.996	1.000	.915	.946
90	—	—	—	—	—	—	.075	.040
Fis		−0.003			−0.004		−0.083	−0.046
Est-3								
N	67	133	4	59	98	17	0	0
125	.044	.060	—	.051	.052	—	—	—
120	.231	.241	.375	.263	.321	.412	—	—
110	.299	.282	.125	.254	.250	.176	—	—
100	.336	.383	.500	.415	.311	.412	—	—
90	.090	.034	—	.017	.066	—	—	—
Fis	0.086	0.090		0.019	−0.062	−0.121		
Sod-1								
N	109	183	6	75	119	29	53	138
120	.092	.052	.083	.113	.088	.034	—	—
100	.908	.948	.917	.887	.912	.966	1.000	1.000
Fis	0.009	−0.055		0.005	−0.097	−0.036		—
6-Pgd								
N	110	182	6	75	118	29	45	138
110	.109	.113	.083	.140	.110	.172	—	—
100	.891	.887	.917	.860	.890	.828	.978	.986
90	—	—	—	—	—	—	.022	.014
Fis	−0.122	−0.017		−0.052	−0.037	−0.208	−0.023	−0.015

Table 2. Continued.

Locus	*An. arabiensis*						*An. gambiae* s.s.	
	V1			V2			V3	
	Endophagic	Exophagic	Endophilic	Endophagic	Exophagic	Endophilic	Exophilic	Endophilic
α-Gpd								
N	110	185	6	76	119	29	52	138
115	—	—	—	—	—	—	.010	.011
100	1.000	.981	1.000	.954	.983	.983	.990	.989
85	—	.019	—	.046	.017	.017	—	—
Fis		−0.019		−0.048	−0.017	−0.018	−0.010	−0.011
Idh-1								
N	113	184	6	75	119	29	46	138
110	.124	.109	.250	.173	.139	.224	.065	.083
100	.858	.864	.750	.780	.857	.759	.924	.906
90	.018	.027	—	.047	.004	.017	.011	.011
Fis	−0.037	0.052		0.072	−0.025	0.262	−0.072	0.076
Mdh-2								
N	112	186	6	75	119	29	53	138
75	.103	.056	—	.120	.046	.069	—	.007
100	.897	.944	1.000	.880	.954	.931	1.000	.993
Fis	0.273**	0.243***		0.012	−0.048	−0.074		−0.007
Got-1								
N	103	180	6	75	118	25	48	111
110	.015	.017	—	.007	.008	—	.063	.099
100	.980	.972	1.000	.987	.983	.980	.896	.896
90	.005	.011	—	.007	.008	.020	.042	.005
Fis	−0.016	−0.022		−0.010	−0.013	−0.020	0.131	−0.014
Got-2								
N	102	180	6	75	120	22	52	107
185	—	—	—	—	—	—	—	.051
120	.034	.028	.083	.040	.050	.091	.067	.089
100	.966	.972	.917	.960	.946	.909	.923	.855
70	—	—	—	—	.004	—	.010	.005
Fis	−0.036	−0.029		−0.042	−0.053	−0.100	−0.047	−0.050

V1, Mulria; V2, Murengeza (Central Imbo); V3, Niabigina (South Imbo). *, significant at $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$. Fis is not given for the V1 endophilic population because $n < 20$.

Table 3. Significant contingency chi-square tests for comparison of allele frequencies between 2 sample groups and significant values of genotypic differentiation (Fst) and their respective values of Fis and Fit

Comparison between subpopulations	Significant differences for allele frequencies		Degree of genotypic differentiation			
				Fst	Fis	Fit
Endo and exophagic at village V1	Mdh-2	$P = 0.036$	Mdh-2	0.007*	0.262***	0.268***
An. arabiensis			Odh	0.012***	0.055	0.067
			α-Gpd	0.010**	−0.019	−0.010
Endo and exophagic at village V2	Idh-1	$P = 0.009$				
An. arabiensis	Mdh-2	$P = 0.007$	Mdh-2	0.018**	0.068	0.084
			Odh	0.008*	0.142	0.149
Endophagic at different villages V1/V2	α-Gpd	$P = 0.005$	α-Gpd	0.024**	−0.048	−0.024
An. arabiensis			Idh-1	0.008*	0.028	0.035
Endophilic and exophilic at village V3	Mpi	$P = 0.024$	Mpi	0.022***	−0.033	−0.011
An. gambiae s.s.			Got-2	0.010*	−0.058	−0.048

*, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$.

lations because the alleles of *Est-2* appeared just below the most common alleles of *Est-3*. Mahon et al. (1976) described the same phenomenon on starch gel for *An. arabiensis*. *Est-3* showed 7 alleles in the *An. arabiensis* populations. Because the fastest (1) and the slowest (7) alleles were extremely rare, they were pooled with the 2nd fastest and the 2nd slowest allele.

Superoxide Dismutase. The *Sod-1*$^{100/120}$ heterozygote was present only in *An. arabiensis* population. *An. gambiae* s.s. was monomorphic for *Sod-1*^{100}. Alleles bands of the 2nd locus were not clear enough to take into account.

6-phosphogluconate Dehydrogenase. One locus showed 3 alleles: *6-Pgd*^{110} and *6-Pgd*^{100} in *An. arabiensis* and, *6-Pgd*^{100} and *6-Pgd*^{90} in *An. gambiae* s.s.

Glycerol-3-phophate Dehydrogenase. An. gambiae s.s. and *An. arabiensis* showed 1 locus comprised of 2 allozymes, *α-Gpd*^{100} and *α-Gpd*^{85}, in both species.

Isocitrate Dehydrogenase. An. gambiae s.s. and *An. arabiensis* showed 3 alleles for the 1st locus, *Idh-1*^{110}, *Idh-1*^{100}, and *Idh-1*^{90}. *An. arabiensis* was monomorphic for *Idh-2*, whereas *An. gambiae* s.s. from Murengeza (V2) showed 2 alleles (*Idh-2*^{90} and *Idh-2*^{100}), but was monomorphic in South Imbo for *Idh-2*^{100}.

Malate Dehydrogenase. An. gambiae s.s. and *An. arabiensis* were monomorphic for *Mdh-1* and showed 2 alleles *Mdh-2*^{75} and *Mdh-2*^{100} for the 2nd locus.

Aspartate Aminotransferase. Two loci with 3 alleles, *Got-1*^{110}, *Got-1*^{100}, *Got-1*^{90} and *Got-2*^{120}, *Got-2*^{100}, *Got-2*^{70} were observed for both species. *An. gambiae* s.s. showed a 4th allele *Got-2*^{185} at the 2nd locus.

Hexokinase. Hexokinase showed 4 constant bands for each specimen and was considered to have 4 loci.

Genetic Analysis. Samples of *An. gambiae* s.s. in Central Imbo and the endophilic *An. arabiensis* sample of V1 were too small for statistical analysis. Therefore, only 5 samples of *An. arabiensis* from Central Imbo and 2 samples of *An. gambiae* s.s. from South Imbo were analyzed statistically.

The test for independence of loci did not show gametic association among loci in the total *An. arabiensis* population. For *An. arabiensis*, the genotypic frequencies of 11 polymorphic loci were in good agreement with random mating, except for *Mpi* in "V2 exophagic" and for *Mdh-2* in "V1 endophagic" and "V1 exophagic" (Table 2). In the *An. gambiae* s.s. V3 samples, the 8 polymorphic loci also were in good agreement with random mating, except for *Mpi* in the endophilic sample. When the exact significance probability test for small samples was performed, *Mpi* was in Hardy–Weinberg equilibrium but not *Mdh-2* (V1 endophagic, $P = 0.015$; V1 exophagic, $P = 0.013$).

An. arabiensis samples did not differ among villages at any loci. The 4 *An. arabiensis* samples from Central Imbo, grouped according to their feeding behavior, were compared 2 × 2 for the 11 polymorphic loci by chi-square contingency test for allelic frequencies and by *F* statistics for genotypic frequencies (Table 3; Fig. 1). These proportions differed significantly for *α-Gpd* (genotypic and allelic frequencies) and for Idh-1 (genotypic frequencies) between the endophagic samples of both villages. In contrast, the 2 exophagic samples did not show any significant difference. Heterozygotes *α-Gpd* $^{85/100}$ were absent in the endophagic sample at V1, but represented 9% of endophagic genotypes at V2.

A comparison of endo- and exophagic females showed significant differences in allelic and genotypic proportions for *Mdh-2* in both villages. Both homozygotes and heterozygotes carriers of the allele *Mdh-2*^{75} had a significantly greater endophagic behavior than the homozygotes for *Mdh-2*^{100} ($\chi^2 = 8.69$, $P < 0.01$) (Table 4). Genotypic proportions differed also for *Odh* in both villages and for α-Gpd in V1, whereas allelic frequencies differed also for *Idh-1* in V2.

In South Imbo, the allelic and genotypic frequencies of *Mpi* and the genotypic frequencies for *Got-2* differed between exophilic and endophilic

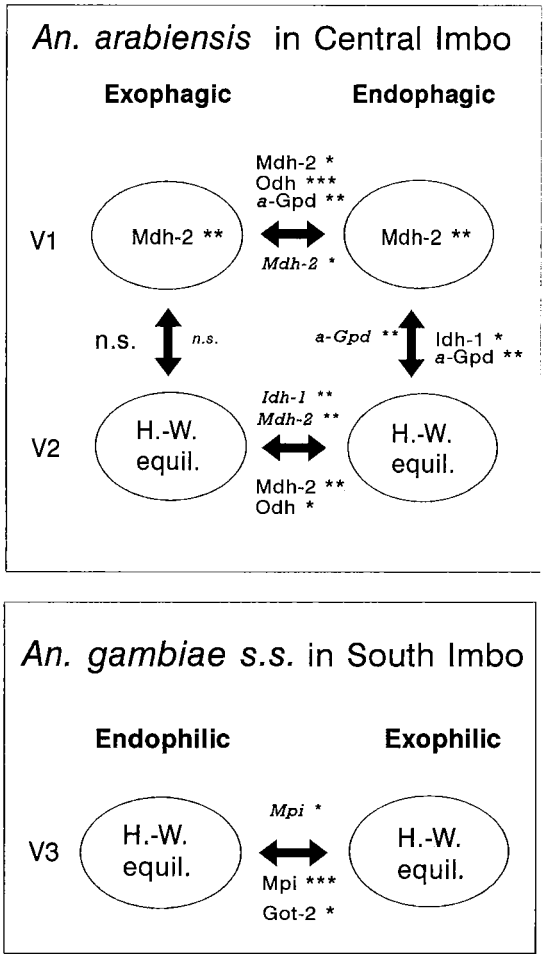

Fig. 1. Loci showing differences in allelic (small italics) and genotypic frequencies (normal font) between 2 subpopulations (arrows). Hardy–Weinberg equilibrium, or departure to it, is mentioned in the circles representing the different subpopulations. *, Significant at $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$; n.s., not significant for all analyzed polymorphic loci.

samples of *An. gambiae* s.s. Homozygotes for Mpi^{120} were about twice more endophagic than the heterozygotes $Mpi^{120130/}$ (Fisher exact: $P = 0.003$) (Table 5).

Nei's genetic distances were 0.154 between *An. gambiae* s.s. and *An. arabiensis* from the Central Imbo and 0.160 between *An. arabiensis* from Central Imbo and *An. gambiae* from South Imbo. Nei's distance between samples of the same species were <0.009.

Discussion

Variation in electromorphs can be compared with those found at chromosomal level (Cianchi et al. 1985), but the usual number of loci analyzed by electrophoresis is limited (15–25 loci). However, electrophoresis has the advantage of being applicable to all specimens, regardless of their physiological and life stage, whereas polytene chromosomes can only be studied on half gravid females. Because liquid nitrogen is available in many veterinary institutions in tropical countries and the liquid air containers have been improved, preservation of material at low temperature has become a minor problem.

Table 4. Distribution of genotype carriers in relation to endo- and exophagic behavior for *An. arabiensis*, Central Imbo

Genotype	Endophagic	(%)	Exophagic	(%)
$Mdh\text{-}2^{75/75}$	6	(67)	3	(33)
$Mdh\text{-}2^{75/100}$[a]	29	(53)	26	(47)
$Mdh\text{-}2^{100/100}$[a]	152	(36)	276	(64)

[a] $\chi^2 = 8.94$, $P < 0.01$.

The *An. gambiae* s.s. and *An. arabiensis* examined in this study did not share alleles for *Odh* and *Mpi* loci. Miles (1979) and Bullini (1984) electrophoretical keys adapted to cellulose acetate worked well on *An. gambiae* s.l. from Burundi. This was supported by the significant positive values of *FIS* for *An. gambiae* s.l. and by the nonsignificant values when species were separated. *Mpi* appears to be a locus as useful as *Odh* in separating these 2 species in Burundi. *Mpi* seems even more effective, because this enzyme has a higher activity than *Odh* and the bands are easier to read. It is interesting that the species composition of the *An. gambiae* complex changes drastically from Central Imbo (2.5% of *A. gambiae* s.s.) to South Imbo (100% of *An. gambiae* s.s.) following the vegetation change, which passes suddenly from the oriental (drier climate) to the Zambesian vegetation.

Genotypic frequencies were generally in good agreement with Hardy–Weinberg expectations, with the exception of the *Mdh-2* locus in the *An. arabiensis* samples from 1 village (V1). Because there was only 1 locus out of 11 polymorphic loci showing a departure from Hardy–Weinberg equilibrium, we may reasonably conclude, as in the study of Hii et al. (1991), that *An. arabiensis* and *An. gambiae* s.s. were each in panmictic situation.

The indoor biting *An. arabiensis* from 2 villages (V1 and V2) differed for 2 electromorph marker (α-Gpd, Idh-1), whereas no difference was seen between exophagic samples in both villages. Smits

Table 5. Distribution of genotype carriers in relation to endo- and exophilic behavior for *An. gambiae* s.s., South Imbo

Genotype	Endophilic	(%)	Exophilic	(%)
$Mpi^{130/130}$	1		0	
$Mpi^{120/130}$[a]	5	(36)	9	(64)
$Mpi^{120/120}$[a]	118	(76)	38	(24)
$Mpi^{115/120}$	1		3	
$Mpi^{115/115}$	0		0	

[a] Fisher exact test, $P = 0.003$.

et al. (1995) showed that *An. gambiae* s.l. human-biting rates were about twice higher in V1 than in V2 and that transmission dynamics of *P. falciparum* in 1993 was quite different in the 2 villages. The main peak of sporozoite inoculation in V1 was observed in April, whereas it occurred in V2 during February. Considering the difference in ecology of breeding sites in these 2 villages, rice fields in V1 and permanent swamps in V2, we were interested in genetic differences between these villages. The difference of alleles distribution seen between endophagic subpopulations (α-Gpd, Idh-1) seems to be an indication of spatial structuring even though the villages are separated by <5 km.

In 2 different villages, endophagic *An. arabiensis* samples differed significantly in their allelic (V1, *Mdh-2;* V2, *Mdh-2, Idh-1*) and genotypic (V1, Mdh-2, Odh, α-Gpd; V2, *Mdh-2, Odh*) proportions from the exophagic ones because of a behavioral structuring. We then could expect a higher sporozoite index in the endophagic population as a result of increased human–vector contact. However, it was difficult to confirm a significant difference in sporozoite indices between the endophagic and exophagic anophelines because of their low observed values (0.1–1.2%) (Smits et al. 1995). In Kenya a 2-fold difference in *Plasmodium* infection rate was found for the standard homokaryotype 2L of *An. arabiensis* compared with the inverted homokaryotype 2La, but this was apparently not related to host-seeking behavior (Petrarca and Beier 1992).

Behavioral structuring occurs also in *An. gambiae* s.s. in South Imbo where indoor resting and egressing mosquitoes showed significant differences in electromorph (*Mpi*) and genotypic frequencies (*Mpi, Got-2*), whereas in Thailand Hii et al. (1991) found differences at 2 loci (*Est-3* and *Idh-2*) in *An. balabacensis.*

Asymmetrical distribution of the various genotypes of *Mdh-2* for *An. arabiensis* and of *Mpi* for *An. gambiae* s.s. in relation to the sampling methods supports the hypothesis of behavioral structuring; i.e., the choice of biting or resting place is associated with genetic variation. Similar results were obtained in West Africa (Coluzzi et al. 1979), where *An. arabiensis* carriers of certain inversions on chromosome 2 have contrasted propensities to bite humans and rest indoors. However, in East Africa no differences in inversion frequencies for both species were observed in the samples collected with different methods but inverted homokaryotypes 2Rb of *An. arabiensis* were more prone to move indoors after feeding outdoors on cattle (Petrarca and Beier 1992).

Nei's genetic distance between *An. gambiae* s.s. and *An. arabiensis* was of the same range of magnitude as calculated by Cianchi (1983) (0.143–0.172) or by Bullini (1984) (0.13). Genetic distances among *An. arabiensis* samples were low (<0.009) compared with West African cytotypes of *An. gambiae* s.s. (0.015–0.030) (Cianchi et al. 1983), which confirms the lack of reproductive isolation.

This study illustrates that populations of *An. arabiensis* and *An. gambiae* s.s. can be structured genetically at spatial and behavioral levels. This does not necessarily imply assortive mating. Genotypes associated with preferential behavioral patterns may explain the occurrence of behavioral resistance that has been described in different control programs after indoor spraying (Muirhead-Thomson 1960). However, if the vector populations are panmictic, as suggested by the current results, the selection of exophilic or exophagic vectors, or both, is not likely to occur.

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