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Full Length Research Paper

Studies on the morphology and compatibility between *Schistosoma hæmatobium* and the *Bulinus sp*. complex (gastropoda: planorbidae) in Cameroon

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A description is given of the morphological variation of the shell, the radula features and the copulatory organ of *Bulinus* sp. (2n=36) from four populations in the western Cameroon crater lakes. To assess the role of diploid snails belonging to the *Bulinus natalensis/tropicus* complex in the transmission of urinary schistosomiasis in Cameroon, the relation between *Bulinus* sp. (from four Cameroon crater lakes) and *Schistosoma haematobium* (from three transmission foci) were studied. *Bulinus* sp. in the present study refers to the diploid snail (2n=36) tentatively identified as *Bulinus natalensis* or as *Bulinus tropicus* in the Cameroon crater lakes. The percentage infection of snails challenged ranged from 03.33 to 06.00% for Nchout Monoun population and from 01.85 to 04.76% for Monoun Ngouondam population. No progeny from Petponoun-East and Petponoun-West were experimentally successfully infected with *S. haematobium*. All the 351 snails dissected were euphallic. Previous malacological surveys revealed the absence of *Bulinus* sp. naturally infected with human schistosomes. These results suggested that *Bulinus* sp. was not susceptible to infection with *S. haematobium* in the Cameroon Western highland crater lakes. These observations justify the absence of transmission foci (for urinary schistosomiasis) in this area.

Key words: *Bulinus,* compatibility, shell, morphology, schistosomes, Cameroon.

INTRODUCTION

In Central Africa, two species of *Bulinus natalensis/tropicus* complex are currently recognised to occur: *Bulinus truncatus* (Audouin 1827) (a tetraploid species, 2n=72) is the most widely distributed while *Bulinus* sp. (a diploid species, 2n=36) is mainly distributed in the western highland crater lakes (Same-Ekobo, 1984; Mimpfoundi and Greer, 1990; Takougang, 1990; Brown, 1994; Mimpfoundi and Ndassa, 2001). From previous observations on snails belonging to the *B. natalensis/tropicus* complex in Cameroon (Mimpfoundi and Greer, 1990), some populations were reported as being euphallic (presence of a normal copulatory organ) and diploid (2n=36) but not naturally associated to *Schistosoma haematobium* transmission. These populations occurred mainly in habitats located 1000 m above the sea level. The report of these diploid snails was first confirmed by Mimpfoundi and Greer (1990). They were tentatively identified as *Bulinus natalensis* (Kuster, 1841), a species previously thought to be limited to eastern and western Africa (Brown, 1980; Brown et al., 1991; Mimpfoundi and Ndassa, 2001). From preliminary studies on shell morphology and anatomical features, it appears that specimens from different sites (Nchout Monoun, Monoun Ngouondam, Petponoun-East, Petponoun-West and Monoun and Njindoun), varied in shell morphology. These observations led to thinking that more than one taxon might be present in the area. This hypothesis is supported by report in western Cameroon highland, of *Bulinus tropicus* by Wright (1965) and Takougang (1990); *Bulinus truncatus rholfsi* and *Bulinus umbilicatus* by Same-Ekobo (1984); *B. natalensis* by Mimpfoundi and Greer (1990) and Mimpfoundi and Ndassa (2001). It is known that shell characters are of only limited value for identification of species belonging to the genus *Bulinus* Müller. In contrast, early species

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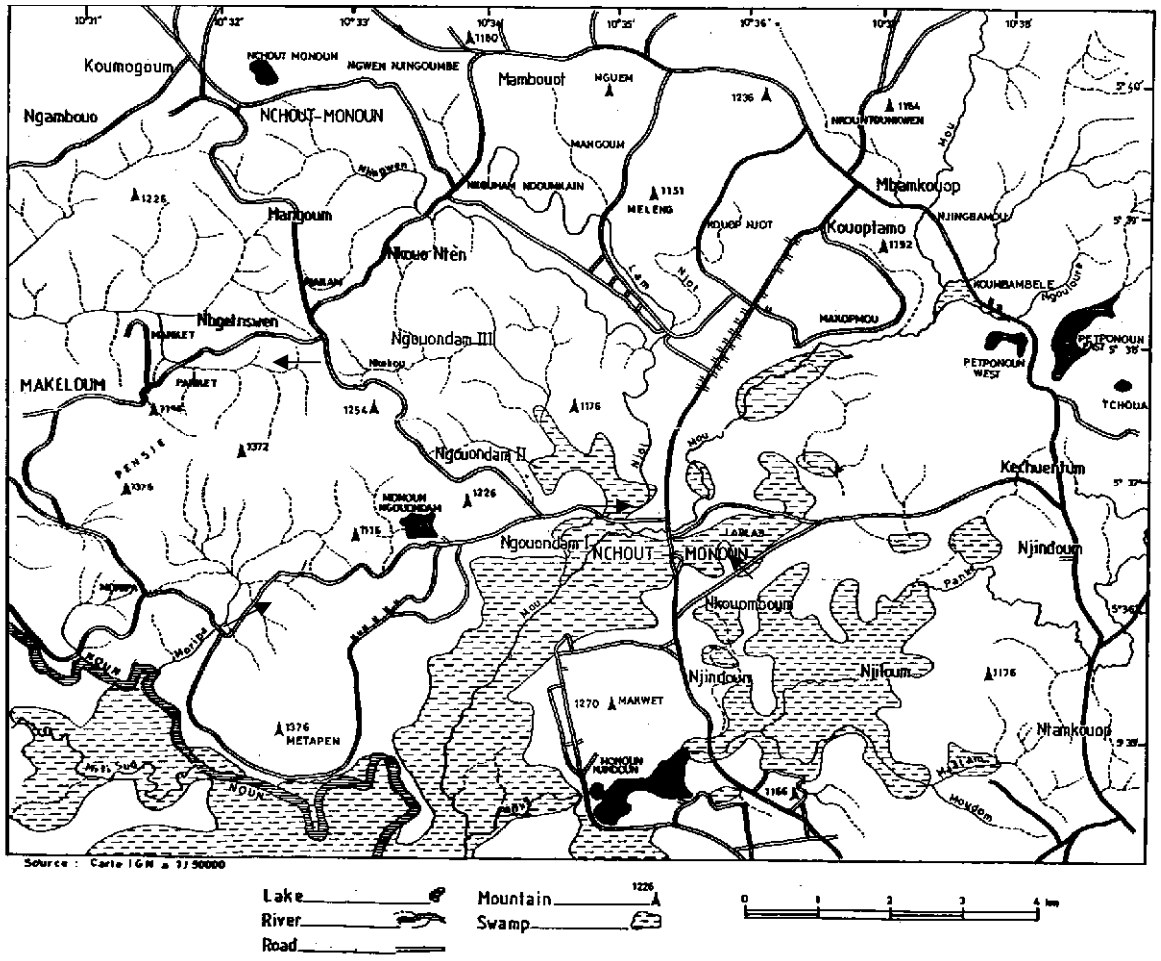

Figure 1. Map of the Noun plain area showing the location of the four sampling sites (Nchout Monoun, Monoun Ngouondam, Petponoun-East and Petponoun-West lakes).

descriptions were based solely on shell characters. Otherwise, malacologists use different methods involving a variety of characters such as shell's features, radula, anatomy, egg-mass morphology, protein electrophoresis, isoelectric focusing immunological methods and susceptibility to identify snails.

In Africa, a wide range of species of *Bulinus* Müller have been reported to act as intermediate hosts for schistosomes (Southgate et al., 1985; 1989; Brown, 1994). *B. natalensis* has been reported to serve as the experimental intermediate host for *S. haematobium* (Lo et al., 1970; Mandahl-barth et al., 1976; Frandsen, 1979) in eastern and southern Africa, and is considered to be involved in natural infections with *Schistosoma bovis* (Graber and Daynes, 1974; Southgate et al., 1985; 1989). It has been established that prior infection of *B. tropicus* and *B. natalensis* with amphistomes is necessary for these snail species to become susceptible to *S. haematobium* (Southgate et al., 1989). Presently, no data is available on experimental infection of diploid *B. natalensis/tropicus* complex species reported in Cameroon.

This study involves two objectives: (1) the use of the shell characters, the radula and the copulatory organ for the morphological description of *Bulinus* sp. (a diploid species, 2n=36) from four sites in the Western Cameroon area; (2) the comparison of snail-parasite compatibility using isolates of *Bulinus* sp. from Western Cameroon and parasites strains (*S. haematobium*) from three allopatric areas in Cameroon which differed in endemicity for urinary schistosomiasis: Bessoum situated downside the Lagdo dam in the northern Cameroon; Loum and Mbanfan situated in the western province of Cameroon.

MATERIAL AND METHODS

Studied populations

Three hundred and fifty one (351) snails of the *B. natalensis/tropicus* complex were collected in four populations (Nchout Monoun, Monoun Ngouondam, Petponoun-East and Petponoun-West) for the present study (Figure 1, Table 1).

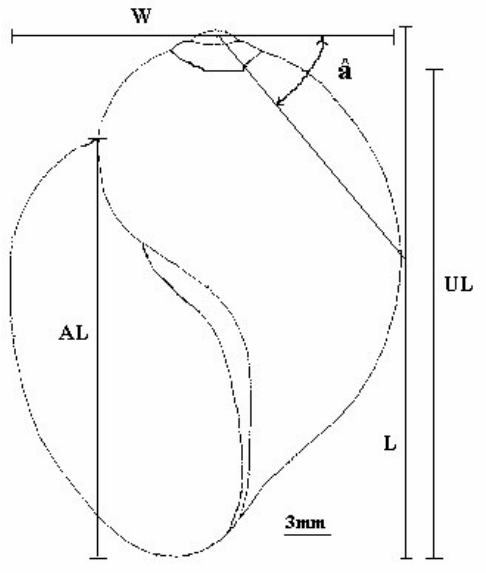

Figure 2. Drawing to show dimensions of the shell illustrated for a specimen of *Bulinus* sp. Shell length (L); aperture length (AL); ultimate whorl length (UL); shell width (W) and apical angle (â). Scale bar= 3mm.

Study of shells

Prior to measurements, the shell was immersed in a saturated oxalic acid aqueous solution. A small tooth brush was used to remove ferruginous deposit and foreign matters on the shell. Damaged shells were removed from samples submitted to examination. Measurements were made using a WILD M5A stereomicroscope equipped with an attached drawing tube at a magnification 12x. Variations were assessed for seven characters (Figure 2): length (L); width (W); length of aperture (AL); length of body whorl (UL); length of spire (SL= difference between length of shell and aperture); number of whorls (NT); and apical angle (â).

Copulatory organ and radula preparation

Each snail was dissected according to Kristensen and Frandsen (1998). Prior to the dissection, snails were narcotised in a saturated solution of menthol. The head region was opened by an incision to remove the penial complex and the radula (from the buccal mass) for preparation. The parameters monitored for the penial complex were the presence (euphallic) or absence (aphallic) of the copulatory organ. For the radula, we noted the shape of the first lateral teeth on both sides of the central tooth of each radula.

Exposure to schistosomes

Snail infection experiments were conducted in the General Biology Laboratory of the University of Yaoundé I. Snails from each of the areas under study were collected and bred in order to obtain the F1 (first generation of offspring from wild snails). *S. haematobium* eggs were obtained from infected urine taken from primary school children who manifested haematuria when tested with “Medi-test Combi 7” for urinalisis (Machery-Nagel, Postfach 101352-D-52313 Düren, Germany). Patients found infected with schistosomes were provided with a curative dose of praziquantel. The schistosome eggs were concentrated by sedimentation in normal physiological saline 0.9%, and then they were hatched as described by Njiokou et al. (2004). Four-weeks-old F1 generation snails were exposed individually to 5 miracidia of *S. haematobium* as described by Combes (1985).

Snails were observed periodically for the presence of *S. haematobium* cercariae from day 28 post-exposure to miracidia. Under a WILD M3Z stereomicroscope compound, the snails were checked for cercariae shedding after an hour of exposure to an artificial illumination. At day 55 post-exposure, all the surviving snails were crushed between two glass-slides and examined to determine the presence of larvals of schistosomes. The snails found with larval stages, were classified as infected with schistosomes. The parameters monitored in the snail room were snail mortality and infection (daily until 6 weeks post-exposure). Snails were fed with fresh lettuce. The water was changed three times per week in order to keep clean water in the aquarium.

Data analysis

The mean, range and standard deviation were estimated for each shell characters within each putative population using the statistical package EPI-info 2000. The mean shell characters among populations were compared using one way analysis of variance (ANOVA) with Post Hoc Newman-Keuls procedure. The one way ANOVA test was performed on continuous shell characters using the statistical package SPSS version 10.1 for Windows. Differences between groups were considered significant at $p<0.05$. The infection rates were estimated by calculating the ratio between the number of infected snails and the number of surviving snails.

RESULTS

Morphometrics

The parameters of shells from the studied sites presented variations (Table 1). Significant differences were detected when the four populations were compared ($F=138.359$; $p<0.05$). But the apical angle (â) in Nchout Monoun and Monoun Ngouondam did not differed significantly ($p=0.957$); so were Petponoun-East and Petponoun-West populations ($p=0.067$). For the spire length (difference between shell length and aperture length), populations from Nchout Monoun and Monoun Ngouondam showed no significant difference ($p=0.226$). The number of whorl did not differ significantly ($p=0.114$) between Nchout Monoun and Petponoun-West populations.

Copulatory organ and radula

All the 351 snails examined on dissection, were euphallic. Six specimens of snails from Petponoun-East presented abnormal penial complex, particularly characterized by the atrophy of the penial sheath and the preputium. The shape of the mesocone (middle cusps) on the first lateral tooth presented a variation. The central tooth showed two cusps of the same size and was relatively smaller, compared to the lateral tooth.

Table 1. *Bulinus* sp. (2n=36) from Cameroon. Measurements of mean shell continuous characters.

Origin of shell	No. of shells examined	â (^{0}C)	L (mm)	UL (mm)	AL (mm)	W (mm)	SL (mm)	N_T
Petponoun-East	109	$40^0$09	11.24	10.54	8.62	8.91	2.61	3.36
		($4^0$13)	(1.05)	(0.97)	(0.86)	(1.02)	(0.65)	(0.26)
Nchout Monoun	82	$48^0$77	7.11	6.71	5.79	5.47	1.30	2.96
		($2^0$97)	(0.87)	(0.81)	(0.66)	(0.60)	(0.42)	(0.35)
Monoun Ngouondam	80	$48^0$80	5.71	5.31	4.28	4.53	1.43	2.5
		($4^0$25)	(0.85)	(0.76)	(0.64)	(0.71)	(0.46)	(0.50)
	80	$41^0$12	9.54	8.94	7.46	7.87	2.07	3.05
Petponoun-West		($3^0$60)	(1.32)	(1.26)	(0.96)	(1.05)	(0.64)	(0.47)
Total	351							

Apical angle (â); length (L); body whorl length (UL); aperture length (AL); Width (W); spire length (SL= difference between length of shell and aperture) and number of whorls (N_T).
Values in brackets indicate standard deviations.

Table 2. Mesocone shape in the populations of *Bulinus sp.* (2n=36) from Cameroon. Numbers refer to percentage of mesocone type.

Origin of snail	No. of radula examined	Shape of mesocone		
		Non angular (%)	Intermediate (%)	Angular (%)
Petponoun-East	22	12.35	29.65	58.00
Nchout Monoun	31	17.78	06.57	75.65
Monoun Ngouondam	18	09.00	38.82	52.18
Petponoun-West	25	16.00	39.00	45.00

Meanwhile, three radula from Nchout Monoun presented the central tooth with only one single cusps. The first lateral tooth was classified as angular, intermediate or non angular. The shapes of the mesocones in the four studied populations were predominantly angular (above 45.00% angular mesocones in each population) (Table 2).

Exposure to schistosomes

The infection rate was similar to those observed in the other relevant studies on diploid snails (Brown et al., 1971, 1991; Chingwena et al., 2002a,b). In total, the compatibility status of four populations of *Bulinus* sp. was determined: 209 from Petponoun-East, 257 from Nchout Monoun, 287 from Monoun Ngouondam and 214 from Petponoun-West (Table 3). The percentage infection was calculated from the proportion of surviving snails found to be shedding cercariae. The distribution of populations whose progeny were experimentally infected is shown in Figure 1. Although the majority of individuals were refractory to infection, some snails from Nchout Monoun and Monoun Ngouondam were compatible with *S. haematobium*. Percentage infection of survivals ranged from 3.33 to 6.00% for Nchout Monoun and from 1.85 to 4.76% for Monoun Ngouondam (Table 3). No progeny from Petponoun-East and Petponoun-West were experimentally infected with *Schistosoma haematobium*.

DISCUSSION

The present results clearly show that the four populations are morphologically different, but *Bulinus sp.* from Nchout Monoun seems closely related to *Bulinus sp.* from Monoun Ngouondam and both differ significantly from populations sampled in Petponoun-East and Petponoun-West. In the four populations, snails are diploid and euphallic. A genetic difference was found for the allozyme migration pattern of nucleoside phosphorylase (NP), with a fast migrating allele in the Nchout Monoun and Monoun Ngouondam samples (Mimpfoundi and Greer, 1990) correlated to the morphological differentiation reported in the present study. According to shell attributes, chromosome number and euphallic genital system, we already came to the conclusion that *Bulinus sp*. from Nchout Monoun seems closely related to the *B. natalensis* (Mimpfoundi and Ndassa, 2001); but differences found between these

Table 3. Results of exposing the *Bulinus* sp. (2n=36) bred from snails at various sites of different geographical isolates of *S. haematobium* in Cameroon.

Origin of:		Number of snails :				
Snails laying the eggs from which the exposed snails developed	***S. haematobium* used (water collection type)**	**exposed**	**surviving**	**Infected**	**Infected nails surviving up to day 55 post-exposure**	**Mortality (%)**
Petponoun-East (CL)	Bessoum (I)	28	13	0	0	53.57
Petponoun-East (CL)	Bessoum (I)	56	29	0	0	48.21
Petponoun-East (CL)	Mbanfan (S)	58	26	0	0	55.17
Petponoun-East (CL)	Mbanfan (S)	20	03	0	0	85.00
Petponoun-East (CL)	Loum (S)	33	16	0	0	51.51
Petponoun-East (CL)	Loum (S)	14	08	0	0	42.85
Total		**209**	**95**	**0**	**0**	54.54
Nchout Monoun (CL)	Bessoum (I)	63	50	3	2	20.63
Nchout Monoun (CL)	Bessoum (I)	22	18	0	0	18.18
Nchout Monoun (CL)	Mbanfan (S)	60	12	0	0	20.00
Nchout Monoun (CL)	Mbanfan (S)	34	30	1	0	11.75
Nchout Monoun (CL)	Loum (S)	64	55	0	0	14.03
Nchout Monoun (CL)	Loum (S)	14	12	0	0	14.28
Total		**257**	**177**	**4**	**2**	31.12
Monoun Ngouondam (CL)	Bessoum (I)	35	32	0	0	08.57
Monoun Ngouondam (CL)	Bessoum (I)	27	25	0	0	07.40
Monoun Ngouondam (CL)	Mbanfan (S)	48	42	2	0	14.58
Monoun Ngouondam (CL)	Mbanfan (S)	59	47	0	0	20.33
Monoun Ngouondam (CL)	Loum (S)	62	54	1	1	12.90
Monoun Ngouondam (CL)	Loum (S)	56	08	0	0	14.28
Total		**287**	**208**	**3**	**1**	25.52
Petponoun-West (CL)	Bessoum (I)	54	47	0	0	12.96
Petponoun-West (CL)	Bessoum (I)	58	29	0	0	50.00
Petponoun-West (CL)	Mbanfan (S)	54	36	0	0	33.33
Petponoun-West (CL)	Mbanfan (S)	11	07	0	0	36.36
Petponoun-West (CL)	Loum (S)	14	08	0	0	42.86
Petponoun-West (CL)	Loum (S)	23	04	0	0	82.61
Total		**214**	**131**	**0**	**0**	38.78

(I) irrigation canal, (S) stream, (CL) crater lake. Petponoun-East (N= 209); Nchout Monoun (N= 257); Monoun Ngouondam (N= 287); and Petponoun-West (N= 214).

populations and other geographically related populations in the western mountainous volcanic region in Cameroon remains controversial. The absence of migration from one site to another may favour these populations to evolve independently (Brown, 1994). Similar observations have been made between *B. camerunensis* from the type locality Barombi Kotto and the population from Debundsha crater lake, both populations showing a genetic divergence, coupled to morphological differences (Mimpfoundi et al., 2001).

This is the first study carried on the compatibility status of diploid snails belonging to the *B. natalensis*/*tropicus* complex species in Cameroon. After laboratory infections, *Bulinus* sp. (2n=36) reported from Cameroon

crater lakes (Mimpfoundi and Greer, 1990; Takougang, 1990; Mimpfoundi and Ndassa, 2001) have been shown to be able to act as experimental intermediate host for *S. haematobium.*

Our ongoing malacological surveys on diploid *B. truncatus/tropicus* complex have so far failed to find evidences of natural infection with human schistosomes in West Cameroon. Similar results were found on diploid species by Stothard et al. (2001), Mukaratirwa et al. (1998); Chingwena et al. (2002a,b) and Mubila et al. (2002) in Madagascar and Zimbabwe. The present results confirm observations made by Lo et al. (1970), Mandahl-Barth et al. (1976) and Frandsen et al. (1979) on the resistance of most diploid populations within the *B. truncatus/tropicus* complex in Africa (Degrémont, 1973). Preston and Southgate (1994) suspected internal defence mechanism to explain the development of resistance, thought Degrémont (1973) found it dangerous to systematically eliminate any species of the *B. truncatus/tropicus* complex on the self pretext that it is diploid. Moreoever, Degrémont (1973) reported that a diploid species (*Bulinus liratus)*, which is a *B. natalensis*-like snail, has been confirmed as compatible with *S. haematobium* in Madagascar. Expanding upon Degrémont's findings, experiments by Stothard et al. (2001) confirmed that there were populations of *B. liratus* (diploid species) in Madagascar, compatible with *S. haematobium*. The lack of cercarial shedding in the majority of the present snail-parasite combinations confirmed the resistance of *Bulinus* sp. and could therefore, justify the absence of infections due to urinary schistosomiasis in Western Cameroon region. Moreover, the low infection rate (01.87 to 06.00%) observed from snails experimentally challenged using five miracidia, has not been considered indicative of a high level of susceptibility as stated by Wright and Ross (1983) and Stothard et al. (2000). Therefore, it is clearly established that the four populations of *Bulinus* sp. from Cameroon crater lakes, are not susceptible to *S. haematobium.*

Generally, sympatric combinations of snails and parasites produce higher prevalence of snail infections than allopatric combinations (Manning et al., 1975; Mukaratirwa et al., 1996; Stothard et al., 2001; Njiokou et al., 2004). Meanwhile, In natural conditions, numerous factors may influence a successful transmission of miracidia to exposed snails. These factors include the survival of the free living-miracidia, the density of miracidia, the history of exposure and the host-parasites specificity.

The prepatent period in the present study (47-49 days) seems rather long enough compared to the maximum (40 days) observed by Njiokou et al. (2004) between *S. haematobium* from Moutourwa (Far-North Cameroon) and *B. truncatus* from Gounoungou (North Cameroon). This long prepatent period confirm the lowest adaptability of *Bulinus sp.* to strains of *S. haematobium.* Nevertheless, a positive correlation was also observed between the compatibility status and the mortality rate of exposed snails. Populations from Nchout Monoun and Monoun Ngouondam were experimentally infected and presented low mortality rates range (7.40-20.63%); in contrast Petponoun-East and Petponoun-West were refractory to infection and they presented the highest mortality rate (above 33.33%) with the exception of a single sample from Petponoun-West, which presented a mortality rate of 12.96%. The mortality rate is a striking parameter since It shares a slightly greater affinity to the parasite pathogenic pressure upon the snail. Moreover, the snail's intrinsic conditions may also be involved in the mortality rate. Therefore, this parameter is difficult to analyse. Nevertheless, the observed mortality rates in the four studied sites could be attributed to the intrinsic conditions of the snail, considering that the experimental conditions were optimised.

Since no transmission focus of urinary schistosomiasis has been found in the Bamoun Plateaux area (Ratard et al., 1990) the nearest geographical isolate of *S. haematobium* investigated was from Loum, but we failed to find any surviving snail infected by this strain in the present study.

According to Gaud (1955), urinary schistosomiasis had been introduced in Cameroon by populations migrating from Sudan during the XIXth century, but a limited number of transmission foci developed successfully where intermediate hosts were compatible in the equatorial region. The absence of urinary transmission foci in the Bamoun Plateaux is a natural argument confirming the absence of compatibility between our diploid populations and *S. haematobium* from the neighbouring foci.

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