Genetic variability in the compatibilityb etween Schistosoma haematobium and its potential vectors in Niger. Epidemiologicali mplications

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Abstract

A populational study of the compatibility between *Schistosoma haematobium* and its potential vectors has been carried out in the Niger, confronting samples of *S. haematobium* populations from three epidemiologic foci with *Bulinus* populations originating from the same focus (sympatric infection) and with *Bulinus* populations from other foci (allopatric infections). The three transmission foci selected were irrigation canals in ricefields along the Niger river where one finds: *Bulinus truncatus rohlfsi*, *Bulinus globosus*, *Bulinus forskalii* and *Bulinus senegalensis*; temporary pools in the Sahel area where one finds *B. truncatus* and *B. senegalensis*; permanent pools of the "guelta" type in Sahara area where only *B. truncatus* occurs. As a compatibility test, the snail infection test was selected, with particular emphasis on optimising its reliability. Snail-infection experiments showed that *B. truncatus* and *B. senegalensis* are very good potential vectors, with infection rates ranging between 71.5 and 85.9%. *B. globosus* and *B. forskalii*, on the other hand, are totally incompatible. The mean infection percentages in the sympatric and allopatric combinations carried out with the *S. haematobium-B. truncatus* couple were very similar. This character strongly suggests a lack of isolation in schistosome populations and a circulation of the parasite genome through the mobility of infected human populations (Peuls and Touaregs) in Sahel zone. This study, in relation with snail surveys carried out in parallel, shows that the main types of aquatic environments on the Niger act as high risk areas for schistosome transmission.

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Introduction

The compatibility between schistosomes and vector snails appears more and more as an essential point in the ecology of schistosome transmission and in the estimation of the potential risks of extension of the parasitose. The study of this characteristic has given rise to a number of fundamental works in the terminal spined egg schistosome group and especially in the species *Schistosoma haematobium* (Webbe and James, 1972; Wright and Knowles, 1972; Frandsen, 1979; Mutani et al., 1985; Southgate et al., 1985).

In these general studies, the authors were mainly interested in the compatibility of *S. haematobium* and *Bulinus* isolates, either strictly sympatric or from very different geographical origins. Although they brought to light the spectrum of potential vector snails of *S. haematobium* in each of the great bioclimatic African areas (North-West, North-East, Central, East and South Africa), these studies do not consider the problem of the polymorphism of the compatibility of a same parasite-host combination inside a same geographical area.

Moreover, most of the works on compatibility envisaged so far were carried out with isolates of snails and parasites kept in the laboratory, sometimes for several generations. Laboratory isolates, often made up of small samples of individuals (parasites and hosts), reflect only imperfectly the genetic variability of the compatibility. It is also very well known now that maintaining isolates of *S. haematobium* on rodents (hamster, meriones) in the laboratory, leads to a genetic drift in the parasite which affects in particular the infectivity of the miracidium. Schistosome-snail compatibility results obtained from isolates of schistosomes kept on rodents, which is the case in most of the works carried out so far, are therefore undoubtedly leaned over in relation to real values.

Since our earliest comparative studies on the dynamics and genetics of *S. haematobium* populations several years ago in the most representative transmission sites of the Niger, we have continued to study Schistosome-*Bulinus* compatibility directly in the field, using samples of parasites and snails from natural populations for each experiment. This study thus represents one of the first truly populational approaches to the schistosome-vector snail compatibility character, carried out of the *S. haematobium-Bulinus* model.

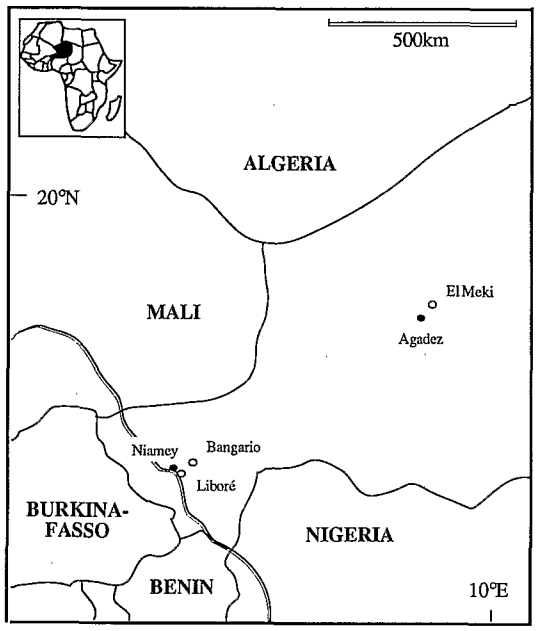

Fig. 1 Sketch-map of the study areas in Niger

Materials and methods

Study area

An epidemiological study carried out previous to our own has shown that the transmission foci of urinary schistosomiasis in the Niger belong to three main epidemiologic foci (Fig. 1): irrigation canals in hydroagricultural complexes, temporary pools in the Sahel area and permanent pools of the "guelta" type in the Sahara area.

Hydroagricultural complexes, essentially for rice fields, are found mainly in the valley of the river Niger. Snail populations prosper preferentially in the secondary and tertiary canals in which we found the following species: *Bulinus truncatus rohlfsi*, *Bulinus globosus*, *Bulinus forskalii*. *Bulinus senegalensis* is also encountered in this area, but the numbers are very small: it lives only in very small pools, formed near the canals during the rainfall season. The study area we selected for this type of transmission focus is situated at 15 km to the south of Niamey, in the Libore district, on the left bank of the river.

The transmission foci represented by temporary pools are disseminated throughout the sahel zone of the Niger. These pools, variable in size (from a few m^2 to several hectares), are often found in the beds of fossil-valley (dallols) and of temporary rivers (koris). They remain full of water between 5 weeks and 9 months, from July. The pools with a short lifespan are colonized during the rainfall season by *B. senegalensis*. In the pools with a longer lifespan (more than 7 months), *B. senegalensis* is replaced by *B. truncatus rohlfsi*, which remains until the drying out period. We selected, as representative of temporary pools, the pools in the Bangario area, localized in the Tabla district, at 110 km to the north east of Niamey.

Permanent pools are localized in the Air mountains, in the north of the Niger. These pools, called "gueltas" by the locals, of a few hundred meters in surface, are continuously suplied by resurgences. Human populations often concentrate round them. Very dense populations of *Bulinus truncatus rohlfsi* live in these pools all the year round. Very small populations of *B. senegalensis* can be found, only during rainfall season, in ephemeral paddles situated in the bed of the "koris". Our study area for this type of transmission focus is situated at the north of Niger, in the El Meki area, at 200 km to the north-east of Agadez city.

Intermediate hosts

The bulinids snails used in experimental infections are a random sample of young individuals (shell size between 2 and 4 mm high), taken exclusively from the first generation descendants of natural populations of parents. The parents were constantly renewed to meet the requirements of our experiments.

Parasite strains and infection procedure

Eggs of *S. haematobium* are collected, in each of the three transmission sites, from the urines of 20 bilharzian children, chosen in the most infected age range (between 10 and 15 years old). Eggs started hatching 48 hours at later after being collected.

The Bulinids species are individually exposed to 5 miracidia for 24 h, in plastic vials containing 0.5 ml of spring water. Then the snails are maintained in batches of 25, in culture Petri dishes of blue-green algae of the *Nostoc moscorum* species, produced by the Liang and Van der Shalle (1975) technique. When the all the algae thallus has been consumed (about 6 days later), the snails are placed in batches of 25 in flat tanks with 3 litres of spring water and fed with dry lettuce and *Nostoc moscorum* thallus. The water temperature in the tanks is maintained at + 26 °C for the whole of the experiment. The snails are examined for emerging cercariae between the 30th and the 45th day postexposure.

Experimental conditions were chosen to enable us to confront each *S. haematobium* population with *Bulinus* populations from its own area (sympatric infections) and with *Bulinus* populations from other areas (allopatic infections).

Results

S. haematobium-Bulinus sp. compatibility tests were carried out on a total of 1997 *Bulinus*, including 1253 *B. truncatus*, 397 *B. senegalensis*, 248 *B. globosus* and 99 *B. forskalii*.

In Tables 1, 2 and 3, we give the results of all the individual infection experiments carried out with miracidium populations of *S. haematobium* from Libore, Bangario and El Meki respectively.

In Fig. 2, we show the mean infection percentages for each combination tested, calculated from individual experiments.

From the combined results in Fig. 2, it appears that two species (*B. forskalii* and *B. globosus*) are incompatible or almost totally incompatible with all three populations of *S. haematobium* (the infection rate was respectively 0% and 0 to 3.1%). On the other hand, the two other species tested, *B. truncatus* and *B. senegalensis*, appear to be extremely compatible, the infection rate ranging from 72.7 bo 85.9% in the case of *B. truncatus* and from 71.5 to 85.7% in the case of *B. senegalensis*.

If we consider the compatibility between *S. haematobium* and *B. truncatus* separately for each of the three *S. haematobium* populations, we can make the following observations:

- the compatibility obtained in allopatric combinations are very similar to this obtained in sympatric combinations. At the scale of the bioclimatic zone studied (the Niger Sahel zone), it seems that there is no genetic variability in the *S. haematobium-B. truncatus* compatibility between the different possible combinations of hosts and parasites of varied origin;

Table 1 Individual experimental infections with *S. haematobium* from Libore area. Results of exposing *Bulinus* spp. from three schistosome transmission foci to 5 miracidia hatched from eggs found in human urine

	Date of infection	No. exposed	No. surviving	No. surviving infected	% surviving infected
B. truncatus	87-02-12	43	31	26	83.9
Liboré	87-02-20	47	38	28	73.7
	87-04-28	59	18	14	77.8
	87-07-13	29	8	8	100
	88-01-12	94	60	52	86.7
	88-02-01	100	75	70	93.3
	88-02-08	60	49	37	75.5
B. truncatus	87-01-05	45	35	19	54.3
Bangario	87-02-12	45	20	18	90
	87-02-20	54	25	23	92
	87-03-06	45	18	14	88.8
	88-01-04	16	15	14	93.3
	88-02-01	64	50	27	54
	88-10-17	39	31	26	83.9
B. truncatus	87-02-20	50	23	15	65.2
El Meki	87-03-30	42	32	21	65.6
	87-04-13	48	27	26	96.3
	88-02-21	127	82	70	85.4
	88-08-08	60	20	15	75
B. senegalensis	87-09-28	53	42	21	50
Bangario	87-10-05	14	0		
	87-11-16	25	12	9	75
	88-05-17	65	20	16	80
	88-10-10	70	42	37	88.1
B. forskalii	87-04-06	42	38	0	0
Liboré	87-07-01	41	14	0	0
	87-09-21	37	22	0	0
B. globosus	87-04-28	61	43	0	0
Liboré	87-07-06	26	22	0	4.5
	87-10-05	52	31	2	6.5

Table 2 Individual experimental infections with *S. haematobium* from Bangario area. Results of exposing *Bulinus* spp. from three schistosome transmission foci to 5 miracidia hatched from eggs found in human urine

	Date of infection	No. exposed	No. surviving	No. surviving infected	% surviving infected
B. truncatus	87-02-04	46	37	22	59.5
Liboré	87-12-15	62	44	37	83.7
B. truncatus	87-02-12	49	39	28	71.8
Bangario	87-02-18	106	60	46	76.6
	87-03-18	28	20	15	75
	87-09-03	80	45	31	68.9
B. truncatus	87-02-04	50	37	30	81.1
El Meki	87-12-15	50	24	22	91.7
	88-07-27	37	24	15	62.5
	88-09-30	55	30	22	73.3
B. senegalensis	87-12-15	40	27	21	77.8
Bangario	88-05-30	37	28	24	85.7
	88-07-12	35	23	22	95.7
	88-10-13	121	61	51	85
	88-10-25	76	51	44	86.3
B. forskalii	88-06-14	50	25	0	0
Liboré					
B. globosus	87-12-15	32	27	0	0
Liboré	88-05-30	35	20	0	0
	88-10-13	70	41	2	4.9
	88-05-30	46	31	0	0

Table 3 Individual experimental infections with *S. haematobium* from El Meki area. Results of exposing *Bulinus* spp. from three schistosome transmission foci to 5 miracidia hatched from eggs found in human urine

	Date of infection	No. exposed	No. surviving	No. surviving infected	% surviving infected
B. truncatus Liboré	87-09-19	84	71	61	85.9
B. truncatus Bangario	87-09-19	88	67	53	79.1
	88-12-02	45	20	18	90
B. truncatus El Meki	87-09-19	116	78	67	85.9
B. senegalensis Bangario	87-09-19	20	10	0	0
	88-12-02	156	81	0	0
B. globosus Liboré	87-09-19	39	33	0	0

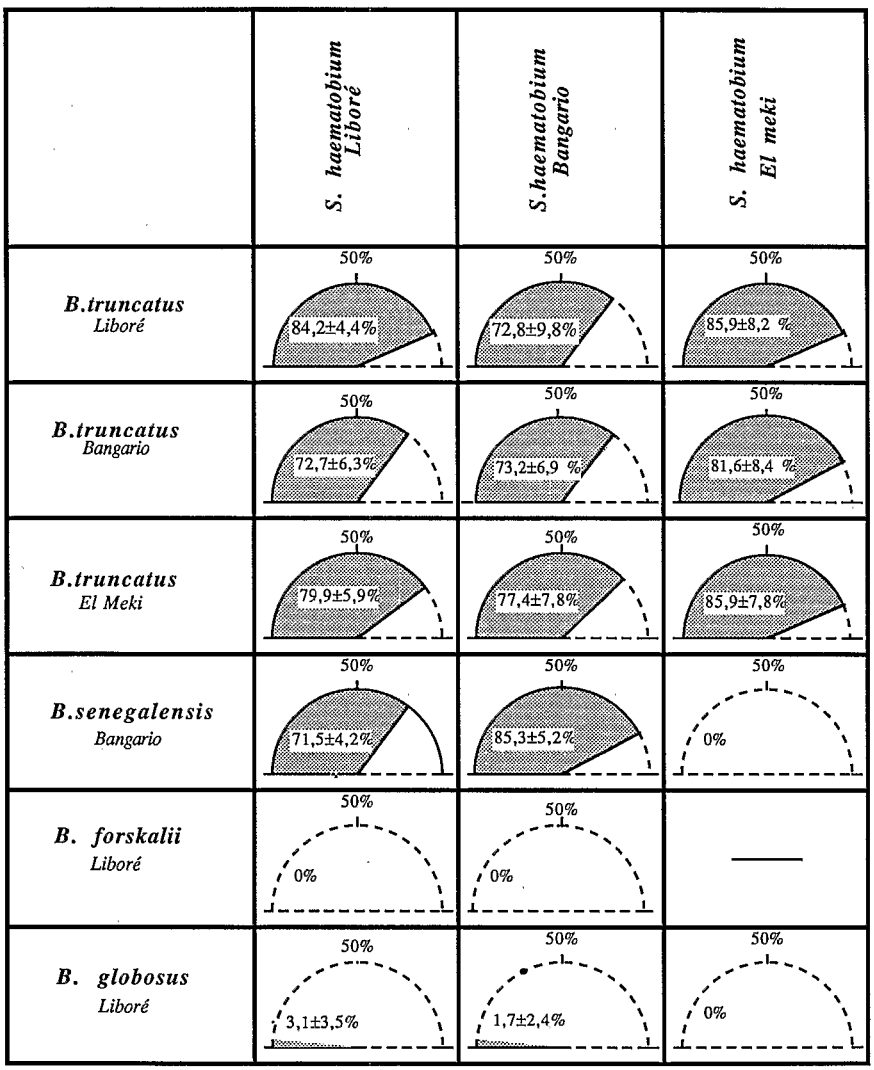

Fig. 2 Snail infection experiments: mean (±SD) of percentage of surviving infected snails. Data from individual experimental infections in Table 1, 2 and 3 are combined

– sympathic infections carried out with each of the three schistosome populations show the same degree of success. There does not seem to be a schistosome population which is more closely adapted to its sympatric population of *B. truncatus*.

The compatibility between *S. haematobium* and *B. senegalensis* has only been studied with the population of *B. senegalensis* from Bangario. *B. senegalensis* is also present in the Libore transmission area, but in such a low density that a statistic significant experiment was not possible. The compatibility levels noted in both compatible combinations are very high and close to those observed with *B. truncatus*. On the other hand, the confrontation of *S. haematobium* El Meki with *B. senegalensis* Bangario reveals a complete incompatibility of the combination.

Discussion

To appreciate the degree of compatibility between schistosomes and snails, a series of different tests, used either together or independently depending on the authors, was set up. Combes (1985) makes a critical analysis of all these tests which he classifies in three categories:

- parasite performance tests (calculating either the percentage of miracidia which penetrate, the percentage of miracidia which develop or the prevalence of experimental infection);
- snail performance test (quantifying the humoral and cellular defence reactions of the Snail);
- system performance test (estimating the amplitude and the length of cercarial production).

The test most frequently used so far is the calculation of the snail experimental infection rate, probably because of the ease of setting in up. Nevertheless, one of us (Jourdane, 1982) has pointed out that it can only be reliable if the experimental conditions are very precisely mastered, which is far from the case in most published works.

The cercarial production test has been used mainly by Webbe and James (1972), Frandsen (1979) and Mutani et al. (1985). Doubtless, the value of cercarial production can constitute an element for appreciating the compatibility that exits between schistosome and snail, but it is obvious that experimental conditions in carrying out the test are very difficult to master.

We put forward (Jourdane, 1982; Jourdane and Xia Mingyi, 1986) a new compatibility test which tests the performance of both the parasite and the host. This test consists in infecting the snails by microsurgical transplantation of sporocysts or miracidia. Any interference due to experimental conditions is kept to an absolute minimum, allowing compatibility to be tested at the truly immunological and populational levels. This test, already used successfully in the *S. mansoni* (Jourdane, 1982) and *S. japonicum* (Jourdane and Xia Mingyi, 1986) models, and which looks very promising for the future, has been adopted and perfected on the *S. haematobium* model in the course of this present work. The results obtained will be presented comparatively in a paper which is in progress.

The snail infection test has been selected in our work taking into account its feasability in field laboratories. This test has been used according to the procedure described above, with particular emphasis on optimising its reliability.

The global results of the compatibility tests in Fig. 2 very clearly show the potential intermediate hosts of *S. haematobium* in the three types of transmission foci in the Niger. *B. truncatus* and *B. senegalensis* proved to be excellent potential vectors, since the mean infection rates obtained for 11 out of the 12 combinations tested lie between 71.5 and 85.9%. The role played by *B. globosus*, a species of the *africanus* group, appears from our results to be negligible or non-existant. Also, our data suggest that *B. forskalii* does not play any role in *S. haematobium* transmission in Niger. The high infection rates of *B. senegalensis* (85.3 and 71.5% obtained with *S. haematobium* populations from Bangario and Libore respectively) confirm recent data revealing the importance of this vector in *S. haematobium* epidemiology in the Sahel zone. Goll and Wilkins (1984) were first to demonstrate the role of this snail in the transmission of *S. haematobium*, in field work carried out in the temporary pools of Senegambian plateau. Southgate et al. (1985), successfully carried out the experimental infection of *B. senegalensis* from Senegal with a strain of *S. haematobium* of the same geographical origin, while two other Senegal species of *Bulinus*, *B. forskalii* and *B. guernei*, have been found incompatible. As concerns the compatibility character, the *S. haematobium* populations in the Niger resemble the north-east and the north-west African populations, which offer very low compatibility or incompatibility with *Bulinus* of the *africanus* group and very high compatibility with *B. truncatus* (Frandsen, 1979; Christensen et al., 1986). Also, they resemble the Senegambian populations which are very compatible whith *B. senegalensis* (Goll and Wilkins 1984; Southgate, 1985).

In all the compatible combinations, compatibility is very high. The values recorded are far above those most often found in the literature, even for combinations between schistosomes and *B. truncatus* of the same geographical origin. It is however worth noting that, in the rare cases when compatibility tests vere carried out using miracidia hatched from eggs found in human urines, the values obtained matched ours (Frandsen, 1979). If we pay particular attention to individual infection experiments (Table 1, 2, 3), we note that, for any given combination, the values observed show a non negligible variability in compatibility. In *S. haematobium* Libore-*B. truncatus* Bangario combination for example, the percentages are distributed between 54 and 93.3%. These differences may result from the difficulties in reproducing the experimental conditions of infection. But they may also reflect the existence of a compatibility polymorphism from one experiment to the next, linked to the genetic diversity of the samples of Schistosomes and Snails confronted. This is particularly true of miracidium populations which may originate from adult populations with different genetic structures, from one sample to the next (for example after re-infection).

At the epidemiological level, the results of our experiments on compatibility are interesting in several respects.

The fact that average infection percentages in the sympatric and allopatric combinations carried out with the *S. haematobium-B. truncatus* couple are not very similar, strongly suggests a lack of isolation in schistosome populations. Since the snail populations are effectively segregated, the circulation of the parasital genome can only occur through the mobility of infected human populations. The strong mobility of some Nomad people in the Sahel zone (Peuls, Touaregs) probably favours considerable genetic mixing by importing the parasite from one infection area to the other. An isolation of schistosome populations inside each infection area would no doubt have favoured host-parasite co-evolutive processes, leading to different compatibility levels, as the selective pressures at work in each area are different.

If we consider the *S. haematobium-B. senegalensis* model, it appears that the allopatric *S. haematobium* El Meki-*B. senegalensis* Bangario combination is totally incompatible. A co-adaptive argument can also be used here: the

exclusive presence of *B. truncatus* in the "guelta" type pools, which represent the only human-water contact points, has no doubt favoured a very strong adaptation of the *S. haematobium* El Meki genome to *B. truncatus*, the only sympatric species of *Bulinus*. This long co-adaptation might no longer allow *S. haematobium* populations from El Meki to develop in other species of *Bulinus*.

Malacological studies carried out in parallel with our work allowed us to learn precisely the dynamics of snail populations in the three types of infection areas in the Niger. The result of this study is that the transmission areas in the Niger are generally stocked whenever they are under water, by at least one of the potential vector species revealed by our compatibility study. Schistosome transmissioin can thus theoretically take place continuously. In irrigation canal and permanents pool types of infection foci, this transmission is possible all year round. On the other hand, in temporary pool infection areas (Bangario), transmission is seasonal in character, nevertheless it may last all the time that they are full of water (from June to February).

It appears from our work that the main types of aquatic environments in the Niger act as high risk areas for schistosome tansmission for at least two reasons: they are continuously colonized by potential vectors of *S. haematobium*; these potential vectors offer a very considerable permissivity (over 70%) not only towards sympatric populations of *S. haematobium* but also towards populations of *S. haematobium* from allopatric transmission areas.

Epidemiological studies are currently being carried out in the Niger on both the Man and Snail compartments, and on the water interface, with a view to modelling the transmission modes of *S. haematobium* in typical infection areas of the Sahel-Saharian zone.

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