Population genetic structure of the schistosome-vector snail *Bulinus globosus*: examining the role of genetic drift, migration and human activities

F. NJIOKOU ‡†, B. DELAY§, C. BELLEC†, E. K. N'GORAN‡, G. YAPI YAPI‡ & P. JARNE§*

‡ Université de Yaoundé I, Laboratoire de Biologie Générale, Faculté des Sciences, BP 812, Yaoundé, Cameroun;

† Laboratoire d'Epidémiologie des Grandes Endémies Tropicales, Orstom, 911 Avenue Agropolis, BP 5045, 34032

Montpellier, France; ‡ Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire, CEMV-01 BP 2597,

Bouaké, Côte D'Ivoire and § Génétique et Environnement, CC 65, Institut des Sciences de l'Evolution, Université

Montpellier II, Place Bataillon, 34095 Montpellier, cedex 5 France

Bulinus globosus, one of the intermediate hosts of the genus *Schistosoma*, is an hermaphrodite freshwater snail species. This species occupies different kinds of environments in Africa, which are generally subjected to large variations in water availability. The mating system of this species is outcrossing, although selfing has been suggested for one population. Here we investigate the genetic structure of populations of *B. globosus* in natural and human-modified environments from Niger and Ivory Coast using protein electrophoresis. Our goal is to analyse the respective role of the environment (genetic drift and gene flow), the mating system and human activities. Our results indicate a low intrapopulation polymorphism. A large differentiation among populations occurs on the scale of the whole study area whereas neighbouring populations can show little differentiation. These results point to the role of genetic drift and gene flow as the main factors shaping the genetic structure, with human activities playing a role occasionally.

Keywords: genetic drift, hermaphrodite, human activity, schistosome, self-fertilization, snail.

Introduction

Three groups of interacting factors influence the genetics of natural populations of tropical hermaphrodite freshwater snails (Jarne & Delay, 1991). (i) The environmental conditions: because the habitats occupied are patchily distributed and water availability varies in time and space (Brown, 1980) there are important variations in population size. These populations therefore experience genetic drift and the genetic consequences of cycles of extinction/recolonization (Slatkin, 1985a; Wade & McCauley, 1988). (ii) The mating system: these snails are self-fertile hermaphrodites and can reproduce both by selfing and outcrossing (review in Geraerts & Joosse, 1984; Jarne *et al.*, 1993), each of which has different genetic consequences (Charlesworth & Charlesworth, 1987). (iii) Parasites: many snail species are intermediate hosts for various trematodes including the human-infecting

schistosomes (the cause of bilharziasis). These parasites have been shown to influence the life-history traits of these snails (Anderson & Crombie, 1984; Minchella, 1985). They may also modify the population genetic structure through their effect on the mating systems of snails (Hamilton *et al.*, 1990; Jarne *et al.*, 1993) and also because resistance to schistosomes is genetically determined (Richards, 1970, 1973).

A metapopulation model taking into account these three groups of factors has been proposed to explain the functioning of hermaphrodite freshwater snail populations (Jarne & Delay, 1991); this model incorporates the effects of genetic drift, gene flow and in some species self-fertilization, to account for the low intra- and interpopulation variability (see Jølnes, 1986; Njiokou *et al.*, 1993) of these species. The metapopulation model can be tested in particular species using allozyme data.

We focus here on the population genetics of the diploid species *Bulinus globosus*, one of the main intermediate hosts of *Schistosoma* in Africa (Brown, 1980).

*Correspondence.

This species is a self-fertile hermaphrodite that preferentially outcrosses (Rudolph, 1983; Rudolph & Bailey, 1985; Jarne *et al.*, 1991), although high selfing rates occur in some populations (Njiokou *et al.*, 1992). *B. globosus* populations are found in a wide variety of habitats (natural or artificial lakes, ponds, rivers, irrigation systems) over many bioclimatic areas (forest, guineo-soudanese or sahelian savannah) (Brown, 1980; Betterton, 1984; Doumenge *et al.*, 1987). Like other tropical gastropod species, natural populations are subjected to drastic environmental variations and regularly experience reductions in size, extinctions and recolonizations (Marti & Tanner, 1988; Woolhouse & Chandiwana, 1989).

B. globosus belongs to the *Bulinus africanus* taxonomic group (Brown, 1980). Electrophoretic studies were first performed for taxonomic purposes. Although not directly concerned with population genetic structure, these studies indicated a reduced

genetic polymorphism in *B. globosus* (Wright & Rollinson, 1979; Jelnes, 1986). Here we intend to identify the main factors affecting the genetic structure of populations of *B. globosus* by studying populations from Niger and the Ivory Coast. We then briefly discuss how human activities could influence the population genetic structure in snails.

Materials and methods

Thirteen populations of *B. globosus* originating from the Ivory Coast and Niger (Table 1 and Fig. 1) were sampled giving a total of 721 individuals and 23 samples as some populations were sampled at two or three different times (Table 1). The genetic analysis was performed by protein electrophoresis following the techniques described in Jelnes (1986) and Pasteur *et al.* (1988). Thirteen enzymatic systems have been analysed coding for 18 presumptive loci, nine of which

Table 1 Country, sampling site, date of sampling, number of individuals per sample (N), mean number of alleles per locus (A), percentage of polymorphic loci (P) and mean observed heterozygosity (H_o) for each population studied

Country	Site	Date (month/year)	N	A	P	H_o
IC	Elevi (a)	11/1988	23	1.1	5.6	0.010
	Elevi (b)	2/1990	28	1.1	5.6	0.018
	Akakronzipris (a)	11/1988	20	1.1	11.1	0.044
	Akakronzipris (b)	5/1989	25	1.2	16.3	0.051
	Akakronzipris (c)	3/1991	18	1.2	11.1	0.049
	Sakiare	11/1988	32	1.1	5.6	0.019
	Man	5/1989	5	1.2	11.1	0.052
	Kan	2/1990	25	1.1	5.6	0.036
	Manikro (a)	5/1989	13	1.1	11.1	0.017
	Manikro (b)	2/1990	30	1.1	5.6	0.019
	Fronan	2/1990	12	1.0	0.0	0.000
	Tiémé	12/1989	21	1.1	11.1	0.034
	Odiénne sud	12/1989	29	1.1	5.6	0.002
	Odiénne nord (a)	12/1989	22	1.1	11.1	0.028
	Odiénne nord (b)	3/1990	29	1.2	11.1	0.036
NI	Liboré8 (a)	6/1989	98	1.1	11.1	0.035
	Liboré8 (b)	2/1990	48	1.1	11.1	0.027
	Liboré8 (c)	6/1991	50	1.1	11.1	0.033
	Liboré9 (a)	6/1989	50	1.1	11.1	0.042
	Liboré9 (b)	6/1991	50	1.1	11.1	0.037
	Daikaina (a)	11/1988	24	1.3	22.2	0.063
	Daikaina (b)	2/1989	31	1.3	22.2	0.072
	Daikaina (c)	3/1991	38	1.3	22.2	0.067
Mean			31.3 (19)	1.1 (0.08)	10.8 (5.64)	0.034 (0.019)

The mean values of N , A , P and H_o are given with their standard deviation in brackets.

IC: Ivory Coast; NI: Niger.

Fig. 1 Sampling sites in Ivory Coast (a) and Niger (b) are indicated by circles. The bioclimatic systems I, II, III and IV (respectively, forest, preforest area, guineo-soudanese savannah and sahelian savannah) are limited by dotted lines. The line going through Ivory Coast (North-South) is a cattle migration trail.

were polymorphic (Table 2): isocitrate dehydrogenase (*Idh*, E.C. 1.1.1.42), phosphoglucumutase (*Pgm*, E.C. 2.7.5.1), esterase (*Est*, E.C. 3.1.1.1), aspartate-amino-transferase (*Aat*, E.C. 2.6.1.1), nucleoside-phosphorylase (*Np*, E.C. 2.4.2.1), mannose-phosphate-isomerase (*Mpi*, E.C. 5.3.1.8), octanol-dehydrogenase (*Odh*, E.C. 1.1.1.73), glucose-phosphate-isomerase (*Gpi*, E.C. 5.3.1.9), hydroxybutyrate-dehydrogenase (*Hbdh*, E.C.

3.1.1.31), α -glycerophosphate-dehydrogenase (α -*Gpdh*, E.C. 1.1.1.8), 6-phosphogluconate-dehydrogenase (*6-Pgd*, E.C. 1.1.1.43), malate-dehydrogenase (*Mdh*, E.C. 1.1.1.37), peptidase B (*PepB*, E.C. 3.4.11).

The allelic frequencies, the proportion of polymorphic loci per population (P), the allelic diversity (A) and the observed heterozygosity (H_o) (Lewontin, 1974) were estimated. A locus was considered as polymorphic when more than one allele was present. When more than one sample was available for a population, we tested the hypothesis that observed changes in allelic frequencies over time can be attributed solely to sampling error and genetic drift using the method developed by Waples (1989). Tests were performed using the software TEMPTTEST (Version 2.2, 1990). As the actual and effective sizes are unknown in the populations we studied, we performed a battery of tests at each polymorphic locus with N_e ranging from 50 to 1000 and N ranging from N_e to $10N_e$ as suggested by Waples (1989). Tests were performed assuming either three or four generations per year. Other assumptions of Waples's procedure are discrete generations and samples drawn randomly from the whole population and also that forces besides stochastic processes such as natural selection, mutation and migration can be neglected.

The fit to Hardy-Weinberg genotypic frequencies was tested for each sample using either Fisher's test, χ^2 or G -test depending on the sample size and the expected genotypic frequencies (Scherrer, 1984). The genetic structure of populations was analysed using Wright's F -statistics (Wright, 1978). F -values were calculated following Weir & Cockerham (1984) using a modified version of Weir (1990)'s program to estimate the variance of F -values with a jackknife procedure. A χ^2 test was used to test for the significance of F -values. The estimation of F -values was first performed on the nine polymorphic loci. We then excluded either the *Est-2* locus because of the presence of a null allele (see below), or the *Np-1* locus because this locus was polymorphic in only one population. The *Pgm-1* locus was excluded because all alleles were fixed in the different populations which leads theoretically to undefined values of the F -statistics. When more than one sample was available for a population, the sample with the largest number of alleles or individuals was retained which results only in a slight increase in the F -values.

The significance levels of tests were adjusted to account for the large number of tests performed following Rice's (1989) procedure.

We tested for the presence of a null allele at the *Est-2* locus. The phenotype at the *Est-2* locus of some individuals in the population Akakronzipris was 80 (see Table 2) whereas other individuals did not show any activity (phenotype 0). All individuals presented a nor-

Table 2 Allelic frequencies at the polymorphic loci

Locus	Est-1			Est-2			Np-1			Hbdh-1			Gpi-1			Idh-1			Pgm-1			Pgm-2			PepB-2					
	100	105	0	80	100	100	100	100	120	60	80	100	100	110	100	120	100	120	70	80	90	100	115	120	60	70	80	90	100	
Sample																														
AKA a	1.000	0.000	0.425	0.575	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.525	0.000	0.475	0.000	0.000	0.000	0.000	0.000	1.000
AKA b	1.000	0.000	0.560	0.440	0.000	1.000	0.000	0.000	0.000	0.000	0.020	0.980	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.640	0.000	0.360	0.000	0.000	0.000	0.000	0.000	1.000
AKA c	1.000	0.000	0.528	0.472	0.000	1.000	0.000	0.000	0.000	0.000	0.028	0.972	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.667	0.000	0.333	0.000	0.000	0.000	0.000	0.000	1.000
ELV a	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.717	0.283	0.000
ELV b	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.393	0.607	0.000
SAK	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.297	0.703	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
MAK a	0.923	0.077	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.154	0.000	0.846	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
MAK b	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.200	0.000	0.000	0.000	0.800	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
MAN	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.300	0.000	0.000	0.000	0.700	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.700	0.300	0.000	0.000	0.000	0.000	0.000	0.000	1.000
TIE	1.000	0.000	0.524	0.476	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.929	0.071	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
ODN a	1.000	0.000	0.795	0.205	0.000	0.864	0.136	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
ODN b	1.000	0.000	0.966	0.034	0.000	0.879	0.121	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.759	0.000	0.241	0.000	0.000	0.000	0.000	0.000	1.000
ODS	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.983	0.017	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
FRO	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
KAN	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.200	0.240	0.560	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
DAI a	0.792	0.208	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.375	0.625	0.250	0.750	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.083	0.729	0.000
DAI b	0.952	0.048	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.387	0.613	0.274	0.726	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.032	0.645	0.000	
DAI c	0.921	0.079	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.408	0.592	0.197	0.803	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.013	0.684	0.000	
LI8 a	0.974	0.026	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.653	0.347	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
LI8 b	0.927	0.073	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.677	0.323	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
LI8 c	0.890	0.110	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.610	0.390	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
LI9 a	1.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.200	0.800	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.580	0.000	0.000	0.000	0.000	0.420
LI9 b	1.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.200	0.800	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.750	0.000	0.000	0.000	0.000	0.250

AKA: Akakronzipris; ELV: Elvi; SAK: Sakiaré; MAK: Manikro; TIE: Tiémé; ODN: Odienne nord; ODS: Odienne sud; FRO: Fronan; DAI: Daikaina; L18: Liboré 8; L19: Liboré 9.

mal enzymatic activity at the *Est-1* locus. The offspring of a crossing of an individual from this population with an individual of phenotype 100/100 from the Daikaina population were either of phenotype 80/100 or 100. The individual from Akakronzipris was then isolated for 2 months which allowed for the allosperm to be exhausted (Rudolph, 1983; Rudolph & Bailey, 1985). Forty-four of its selfed offspring were analysed for their electrophoretic mobility at *Est-2*. The observed frequencies were then compared with the frequencies expected under the hypothesis that the Akakronzipris individual was of phenotype 0/80 using a χ^2 test.

Nei's (1978) genetic distances among populations were calculated. When more than one sample was available for a population, the same sample was used for the calculation of *F*-values (see above) and genetic distances. The matrix of genetic distances was used to build a UPGMA dendrogram using the software BIOSYS (Swofford & Selander, 1989). The correlation between geographical and genetic distances in Ivory Coast and for the whole dataset was tested using a Mantel test as advocated by Smouse *et al.* (1986). Tests were performed using the software GENETIX (Version 0.0, 1993). Levels of significance were calculated from 1000 Monte Carlo samplings. This procedure accounts for the dependence of the entries in each matrix of distance which increases the value of the correlation (Smouse *et al.*, 1986).

Two methods are currently available for estimating the level of gene flow (*Nm*) between populations. Assuming neutrality of the alleles studied and an equilibrium between genetic drift and migration in an infinite island model, *Nm* is related to *F_{st}* (Wright, 1978). Slatkin (1985b) developed an alternative method based on an empirical relationship between *Nm* and the mean allelic frequency of alleles occurring in only one population (private alleles).

Results

Occurrence of a null allele at the *Est-2* locus

Among the 44 selfed offspring of the Akakronzipris individuals, 12 turned out to have no enzymatic activity and 32 were of the phenotype 80 at the *Est-2* locus (with a normal activity at the *Est-1* locus). This does not differ from the expected frequencies under the hypothesis of a 0/80 parental phenotype assuming Mendelian segregation ($\chi^2_{[1]} = 0.11$, n.s.). We thereafter assumed that a null allele was present at the *Est-2* locus, and the allelic frequencies were calculated assuming Hardy-Weinberg equilibrium (Table 2).

Table 3 Test for the stability of allelic frequencies over generations within the same sampling site at polymorphic loci (Waples, 1989)

Sample	Locus	χ^2
AKA a/AKA b/AKA c (2,4)	<i>Est-2</i> <i>Pgm-2</i>	n.s. n.s.
ODN a/ODN b (1)	<i>Est-2</i> <i>Pgm-2</i> <i>Np-1</i>	* when $N_e = N = 50$ ** n.s.
ELV a/ELV b (4)	<i>PepB-2</i>	* when $N_e > 100$
MAK a/MAK b (2)	<i>Hbdh-1</i> <i>Est-1</i>	n.s. n.s.
DAI a/DAI b/DAI c (1,6)	<i>Est-1</i> <i>Gpi-1</i> <i>Idh-1</i> <i>PepB-2</i>	n.s. n.s. n.s. n.s.
LI8 a/LI8 b/LI8 c (2,4)	<i>Est-1</i> <i>Idh-1</i>	* when $N_e = N = 100$ n.s.
LI9 a/LI9 b (6)	<i>PepB-2</i>	n.s.

Sample names are as in Table 2.

The values in parentheses below the population names are the numbers of generations separating the samples for the tests performed.

Only the level at which χ^2 becomes significant is given:

* $P < 0.05$, ** $P < 0.01$ and n.s. = non-significant.

Within-population variability

The allelic frequencies at the nine polymorphic loci are given in Table 2. There is generally one allele at high frequency or even fixed in all populations. Populations from Niger and Ivory Coast can be strictly distinguished on the basis of their electrophoretic patterns at the *Est-2* and *Pgm-1* loci (diagnostic loci). Numerous private alleles occur within populations (Table 2), such as *Pgm-1*⁹⁰ in Elevi or *Np-1*¹²⁰ in Odienné Nord (Ivory Coast), *PepB-2*⁷⁰ in Daikaina and *PepB-2*⁶⁰ in Liboré 9 (Niger). Some other alleles occur only in two populations (Table 2).

The values of *A*, *P* and *H_o* (Table 1) indicate a low variability. The mean number of alleles per locus varies from 1.0 to 1.3 which means that rarely does a locus show more than one allele at the scale of this study. Moreover, the presence of more than two alleles within the same population was detected only twice. The proportion of polymorphic loci goes from zero in Fronan to 22 per cent in Daikaina and the heterozygosity is never higher than 0.072 with a mean value of 0.034. The variability is possibly higher in Niger than in the

Ivory Coast but this could be due to a small number of populations sampled in Niger.

χ^2 values as calculated by Waples' method for the stability of allelic frequencies over generations are given in Table 3. As numerous tests were performed for each locus–population combination, we only give the significance level and, when relevant, the effective size at which the χ^2 value becomes significant. For example, χ^2 differs significantly from zero for large N_e in Elevi (*PepB-2*). Overall, three values of χ^2 turned out to differ from zero (for some values of N_e and N only; see Table 3) out of 15 locus–population combinations.

One out of 38 χ^2 tests performed shows a significant deviation at the 5 per cent level from Hardy–Weinberg proportions, indicating a heterozygote deficiency (Elevi (a), *PepB-2*).

F_{is} values calculated for each locus and all populations significantly differ from zero at three loci (Table 4). The significance of F_{is} at *Np-1* is trivial since this locus is polymorphic in only one population. A true heterozygote deficiency is indicated by significant positive values of F_{is} at *PepB-2* (due to the deficiency of heterozygotes in Elevi; see Table 3) and *Idh-1*. However, F_{is} is low in these two cases. A further point is that all F_{is} values are positive.

Geographical variation

Nei's genetic distances (not shown) vary between 0 and 0.275. Three groups can be distinguished from the

Table 4 Mean F_{is} , F_{st} and F_{it} values per locus and for all loci

Locus	F_{is}	F_{st}	F_{it}
<i>Gpi-1</i>	0.077 (0.209)	0.809*** (8.134)	0.817 (0.145)
<i>Idh-1</i>	0.128* (0.138)	0.813*** (7.602)	0.824 (0.197)
<i>PepB-2</i>	0.280*** (0.139)	0.616*** (5.254)	0.736 (0.101)
<i>Est-1</i>	0.081 (0.115)	0.067*** (0.058)	0.145 (0.122)
<i>Hbdh-1</i>	0.018 (0.096)	0.234*** (0.743)	0.247 (0.073)
<i>Pgm-2</i>	0.069 (0.247)	0.355*** (1.573)	0.434 (0.302)
<i>Np-1</i>	0.394*** (0.189)	0.196*** (0.126)	0.550 (0.264)
Mean	0.144 (0.029–0.257)	0.743 (0.552–0.920)	0.780 (0.595–0.926)

The means are given with their confidence intervals in parentheses and their significance level using a χ^2 test:

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

analysis of the distance matrix (see UPGMA dendrogram in Fig. 2): populations from Niger, Elevi and other populations from Ivory Coast. The correlation between geographical and genetic distances is significantly different from zero (using Mantel's test) for the whole dataset ($r = 0.922$, $P < 0.003$) and among Ivory Coast populations ($r = 0.459$, $P < 0.05$).

F_{st} values (Table 4) are high for the loci *Gpi-1*, *Idh-1* and *PepB-2* which exhibit either alleles diagnostic of groups of populations, or private alleles. F_{st} is significantly different from zero at these loci suggesting highly structured populations. F_{st} decreases when *Est-2* is not taken into account whereas *Np-1* has little influence on F_{st} .

A closer analysis of the spatial distribution of the genetic variability shows two interesting results: (i) Kan (see Fig. 1a) is located between Sakiaré (50 km South) and Manikro (30 km North). Three alleles occur at *Hbdh-1* (60, 80 and 100) in Kan whereas 80 and 100 only occur in Sakiaré and 80 is absent in Manikro. (ii) Liboré8 and Liboré9 (Niger) are separated by about 2 km and located on the same irrigation system (Fig. 3). These populations can be characterized by the presence or absence of one 'private' (relative to the other population) allele at three loci (Fig. 3), both in 1989 and 1991.

Discussion

Within-population variability

Our results indicate that the allozymic polymorphism is low in *B. globosus* measured either as the number of polymorphic loci per population or the number of

Fig. 2 UPGMA dendrogram based on Nei's genetic distances.

Fig. 3 Irrigation system in Liboré and the sampling sites Liboré8 and Liboré9. Water comes from the Niger river at P and the arrows indicate the water flow. 1, 2 and 3 refer, respectively, to the main, secondary and tertiary canals; 60, 100 and 120 represent the alleles observed at the three loci indicated.

alleles per locus. This polymorphism is slightly higher than that observed by Jelnes (1986) in the same species on a sample of 23 populations from the area of the Niger river. However, Jelnes (1986) analysed only eight loci, four of which showed some polymorphism. The mean observed heterozygosity was 0.019. Wright & Rollinson (1979) observed an even lower genetic variability probably because their populations were mainly from laboratory stocks. Another result is the higher polymorphism maintained in Niger than in Ivory Coast populations. However, the low number of populations sampled in Niger precludes any conclusion.

The loss of genetic variability in subdivided populations compared with infinite size populations mainly results from selection or genetic drift (Lewontin, 1974; Slatkin, 1985a). Assuming an island model with no migration, these forces quickly drive all populations to fixation (Slatkin, 1985a). However, there are few examples of such a population structure resulting only from selection (Selander & Ochman, 1983). On the other hand, genetic drift is a major force in subdivided populations experiencing seasonal variation in their size. Such conditions are often met in populations of tropical planorbid snails (including *Bulinus*) as some environments dry up during the dry season, resulting in mass mortality, and are flooded during the rainy season (Brown, 1980; Marti & Tanner, 1988; Woolhouse & Chandiwana, 1989). Our dataset allowed us to test for the effects of stochastic processes

(genetic drift and sampling error). The results indicate that these two mechanisms alone can explain the change in allelic frequencies over years in four locus–population combinations, assuming some conditions on N and N_e . The sample size is probably small enough in some localities (e.g. Odiene (a) and Elevi (a)) for sampling effects to play a major role in the variation of allelic frequencies. With larger sample sizes (e.g. Liboré8), genetic drift can be suspected to explain more fully these variations. However, no significant change in allelic frequencies over generations was shown in many cases. It should also be noted that no test can be performed when alleles are fixed, the most common situation in the present study.

A low level of polymorphism can result not only from the environmental conditions but also from the mating system (e.g. selfing) which can decrease the fixation time of an allele within a population by increasing the homozygosity (see Lewontin, 1974). It has been suggested that selfing results in a quicker decay of the genetic variability in some snail populations (Selander & Ochman, 1983; Mulvey *et al.*, 1988; Jarne & Delay, 1991). Njiokou *et al.* (1993) summarized the observed heterozygosity and the mating system for hermaphrodite freshwater snails. The dataset available, although limited to three genera, indicates that the heterozygosity is lower in selfing than in outcrossing species, as has been found in plants (Hamrick & Godt, 1989).

B. globosus preferentially outcross (Rudolph, 1983; Rudolph & Bailey, 1985; Jarne *et al.*, 1991). The results obtained by protein electrophoresis (Jelnes, 1986; this study) indicate a general agreement with Hardy–Weinberg proportions at the loci studied. The electrophoretic results are therefore compatible with reproduction by outcrossing. The occurrence of private alleles at low frequency (the only one fixed out of seven occurs in the Elevi population suspected to reproduce by selfing; see below) is a further suggestion of outcrossing although genetic drift can produce similar patterns. However, F_{is} values differ from zero at some loci; this can straightforwardly be explained by particular allelic patterns, except for *Idh-1* (Table 4). Nevertheless all F_{is} values are positive which can be interpreted as inbreeding, either some selfing or biparental inbreeding. An alternative explanation is null alleles (Foltz, 1986) whose frequency can be high in *B. globosus* (Table 2). However, this hypothesis is unlikely to explain a general heterozygote deficiency.

The Elevi population showed a significant heterozygote deficiency at its only polymorphic locus *PepB-2* in 1988. This result, and results obtained with other techniques (Njiokou *et al.*, 1992), suggests a reproduction by self-fertilization in this population. However, most *B. globosus* populations outcross and the low

polymorphism observed is rather the consequence of the drastic variations in the environmental conditions.

Geographic variation

The genetic variability is distributed into three groups (namely Niger, Ivory Coast and the Elevi population), as shown on a UPGMA dendrogram (Fig. 2), and also when using factorial correspondence analysis (Njiokou, 1992). At a lower scale, neighbouring populations from within Niger or within Ivory Coast show either a high genetic similarity which can be explained by the existence of (past) gene flow or by a colonization event from a single population or a striking difference at most loci probably due to the prevention of gene flow by human activity.

The distribution of genetic variability in a subdivided population depends mostly on the spatial distribution of populations, their size, the mode and level of gene flow and the processes of extinction/recolonization, assuming neutral variability (Slatkin, 1985a; Wade & McCauley, 1988; McCauley, 1991). Even if geographical distance is not always a good indicator of the genetic distance between populations (Slatkin & Maruyama, 1975), gene flow is a major factor reducing the differences between populations at least at a scale compatible with migration distance. On the other hand, widely separated populations are not likely to be connected by a direct and high gene flow. The three groups of populations in this study are located in three bioclimatic areas. Populations among these groups are therefore isolated by distance and maybe by the difference/boundaries between their habitat, although birds are able to transport snails over large distances (Boag, 1986). The significant correlation between geographical and genetic distances is an expression of the isolation by distance at the scale of the study. The estimations of Nm are low (0.069 and 0.054 by, respectively, F_{st} and Slatkin's method) which could explain the large genetic differences. Nm as estimated by Slatkin's method remains low when populations from the Ivory Coast only are considered. However, when Elevi and Man – the most isolated populations – are removed $Nm = 1.7$. This value is high enough for gene flow to maintain the genetic homogeneity among Northern Ivory Coast populations. Some ecological studies indeed indicate that migration occurs between neighbouring populations by passive transport of individuals or egg capsules along the water flow or as a consequence of flood (Marti & Tanner, 1988; Woolhouse, 1988; Ndifon & Ukoli, 1989).

The gene flow is nevertheless not high enough in the Ivory Coast to explain the situation observed in the Kan/Manikro/Sakiaré area. Three alleles occur in Kan

at the *Hbdh* locus whereas one of the three alleles (not the same) is absent from the other populations. Moreover, *Hbdh-80* occurs in Akakronzipris, a population just South of Kan and Sakiaré (Manikro is the Northern; see Fig. 1). It could be that one allele appeared in one population and is prevented from spreading (at least in a limited period of time) by distance. For example, *Hbdh-80* may be prevented from spreading towards Manikro. An alternative explanation is that these alleles appeared only a short time ago and that they have not had enough time to disperse even with a high human activity, such as irrigation and cattle movements (Fig. 1).

The influence of human activities. We also estimated Nm using Slatkin's method for the three Niger populations. The low values obtained (0.062) show that they are more genetically differentiated than the Ivory Coast populations although the geographical distances separating them is low (respectively, 2 and 100 km from Liboré8 to Liboré9 and Daikaina). Liboré8 and Liboré9 belong to the same irrigation system but are separated by diverging water currents. The allozyme pattern observed cannot be simply explained without knowing the genetic structure of upstream populations (see Fig. 3). However, human activities seem here to influence the genetic structure of populations by preventing gene flow between populations, as has already been shown in another freshwater snail species by Dillon (1988). On the other hand, human activities probably also promote gene flow among populations by connecting previously separated environments, by transplanting snails along cattle migration trails or by creating new environments (e.g. dams). However, colonization and migration events in natural population as well as in human-modified habitats cannot be easily detected in *B. globosus* because of the restricted variability of allozymes.

Our results indicate that drift and migration can be considered as major factors shaping the distribution of the genetic variability at least in the short term. The mating system can play a role in some populations, as well as parasites, but their influence is still poorly understood. Extinction of populations especially after drought occurs and recolonization of vacant environments happens in a short time (Brown, 1980). It is therefore reasonable to assume a metapopulation model for *Bulinus* populations (Jarne & Delay, 1991 and the present data). However, a clearer understanding of the genetic structure of freshwater snail populations and the estimation of the parameters of the metapopulation requires the use of more polymorphic markers than allozymes.

Acknowledgements

We thank M. F. Bosseno for technical help, B. Sellin and F. Mouchet for generously sending snails, J. Goudet, F. Bonhomme and R. Waples for respectively providing us with his modified version of Weir (1990)'s program, GENETIX and TEMPTEST, J. Britton-Davidian and M. Raymond for discussion and comments on the manuscript, and an almost anonymous referee for comments. This work was supported by ORSTOM grants, by HIB program (PIREN-CNRS) and by Institut des Sciences de l'Evolution (CNRS-UM2). This is contribution no. 94-022 of Institut des Sciences de l'Evolution.

References

- ANDERSON, R. M. AND CROMBIE, J. 1984. Experimental studies of age-prevalence curves for *Schistosoma mansoni* infections in populations of *Biomphalaria glabrata*. *Parasitology*, **79**, 63–94.
- BETTERTON, C. 1984. Ecological studies on the snail hosts of schistosomiasis in the South Chad Irrigation project area, Borno state, northern Nigeria. *J. Arid Env.*, **7**, 43–57.
- BOAG, D. A. 1986. Dispersal in pond snails: potential role of waterfowl. *Can. J. Zool.*, **64**, 904–909.
- BROWN, D. S. 1980. *Freshwater Snails of Africa and their Medical Importance*. Taylor & Francis Ltd, London.
- CHARLESWORTH, D. AND CHARLESWORTH, B. 1987. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.*, **18**, 237–268.
- DILLON, R. T. 1988. The influence of minor human disturbance on biochemical variation in a population of freshwater snails. *Biol. Conserv.*, **43**, 137–144.
- DOUMENGE, J. P., MOTT, K. E., CHEUNG, C., VILLENAVE, D., CHAPUIS, C., PERRIN, M. F. AND REAUD-THOMAS, G. 1987. *Atlas of the Global Distribution of Schistosomiasis*. Centre d'Etudes de Géographie Tropicale (C.N.R.S.)/World Health Organization, Presses Universitaires de Bordeaux, Talence.
- FOLTZ, D. W. 1986. Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. *Evolution*, **40**, 869–870.
- GERAERTS, W. P. M. AND JOOSSE, J. 1984. Freshwater snails (Basommatophorans). In: Tompa, A. S., Verdonk, N. H., van den Biggelaar, J. A. M. (eds) *The Mollusca*, vol. 7 (*Reproduction*), pp. 142–207. Academic Press, London.
- HAMILTON, W. D., AXELROD, R. AND TANESE, R. 1990. Sexual reproduction as an adaptation to resist parasites (A review). *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 3566–3573.
- HAMRICK, J. L. AND GODT, J. W. 1989. Allozymes diversity in plant species. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) *Plant Population Genetics, Breeding, and Genetic Resources*, pp. 43–63. Sinauer Associates Inc., Sunderland, MA.
- JARNE, P. AND DELAY, B. 1991. Population genetics of freshwater snails. *Trends Ecol. Evol.*, **6**, 383–386.
- JARNE, P., FINOT, L., DELAY, B. AND THALER, L. 1991. Self-fertilization versus cross-fertilization in the hermaphrodite freshwater snail *Bulinus globosus*. *Evolution*, **45**, 1136–1146.
- JARNE, P., VIANEY-LIAUD, M. AND DELAY, B. 1993. Selfing and outcrossing in hermaphrodite freshwater gastropods (Basommatophora): where, when and why. *Biol. J. Linn. Soc.*, **49**, 99–125.
- JELNES, J. E. 1986. Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae): the West and North African species reconsidered based on an electrophoretic study of several enzymes per individual. *Zool. J. Linn. Soc.*, **87**, 1–26.
- LEWONTIN, R. C. 1974. *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.
- MCCAULEY, D. E. 1991. Genetic consequences of local population extinction and recolonization. *Trends Ecol. Evol.*, **6**, 5–8.
- MARTI, H. P. AND TANNER, M. 1988. Field observations on the influence of low velocities on drifting of *Bulinus globosus*. *Hydrobiologia*, **157**, 119–123.
- MINCHELLA, D. J. 1985. Host life-history variation in response to parasitism. *Parasitology*, **90**, 205–216.
- MULVEY, M., NEWMAN, M. C. AND WOODRUFF, D. S. 1988. Genetic differentiation among West Indian populations of the schistosome-transmitting snail *Biomphalaria glabrata*. *Malacologia*, **29**, 309–317.
- NDIFON, G. T. AND UKOLI, F. M. A. 1989. Ecology of freshwater snails in south-western Nigeria. I. Distribution of habitat preferences. *Hydrobiologia*, **171**, 231–253.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- NJIOKOU, F. 1992. *Génétique et biologie des populations de deux hôtes intermédiaires de schistosomes à oeuf à éperon terminal: Bulinus globosus (Morelet, 1866) et B. truncatus (Audouin, 1827); conséquences épidémiologiques*. Thèse de Doctorat, Université de Montpellier, France.
- NJIOKOU, F., BELLEC, C., BERREBI, P., DELAY, B. AND JARNE, P. 1993. Do self-fertilization and genetic drift promote a very low genetic variability in the allotetraploid *Bulinus truncatus* (Gastropoda: Planorbidae) populations? *Genet. Res.*, **62**, 89–100.
- NJIOKOU, F., BELLEC, C., N'GORAN, K. E., YAPI, Y. G., DELAY, B. AND JARNE, P. 1992. Comparative fitness and reproductive isolation between two *Bulinus globosus* (Gastropoda: Planorbidae) populations. *J. Moll. Stud.*, **58**, 367–376.
- PASTEUR, N., PASTEUR, G., BONHOMME, F., GATALAN, J. AND BRITTON-DAVIDIAN, J. 1988. *Practical Isoenzyme Genetics*. Halsted Press, New York.
- RICE, W. R. 1989. Analysing tables of statistical tests. *Evolution*, **43**, 223–225.
- RICHARDS, C. S. 1970. Genetics of a molluscan vector of schistosomiasis. *Nature*, **227**, 806–810.
- RICHARDS, C. S. 1973. Susceptibility of adult *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *Am. J. Trop. Med. Hyg.*, **22**, 748–756.
- RUDOLPH, P. H. 1983. Copulatory activity and sperm production in *Bulinus globosus* (Gastropoda: Planorbidae). *J. Moll. Stud.*, **49**, 125–132.

- RUDOLPH, P. H. AND BAILEY, J. B. 1985. Copulation as females and use of allosperm in the freshwater snail genus *Bulinus* (Gastropoda: Planorbidae). *J. Moll. Stud.*, **51**, 267-275.
- SCHERRER, B. 1984. *Biostatistique*. Gaëtan Morin, Quebec.
- SELANDER, R. K. AND OCHMAN, H. 1983. The genetic structure of populations as illustrated by molluscs. In: Rattazzi, M. C., Scandalios, J. G. and Whitt, G. S. (eds) *Isozymes: Current Topics in Biological and Medical Research*, vol. 10, pp. 93-123. A. R. Liss, New York.
- SLATKIN, M. 1985a. Gene flow in natural populations. *Ann. Rev. Ecol. Syst.*, **16**, 393-430.
- SLATKIN, M. 1985b. Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- SLATKIN, M. AND MARUYAMA, T. 1975. The influence of gene flow on genetic distance. *Am. Nat.*, **109**, 597-601.
- SMOUSE, P. E., LONG, J. C. AND SOKAL, R. R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.*, **35**, 627-632.
- Swofford, D. L. AND SELANDER, R. B. 1989. BIOSYS-1. *A computer program for analysis of allelic variation in population genetics and biochemical systematics*. Illinois Natural History Survey, Champaign, Illinois.
- WADE, M. J. AND McCauley, D. E. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995-1005.
- WAPLES, R. S. 1989. Temporal variation in allele frequencies: testing the right hypothesis. *Evolution*, **43**, 1236-1251.
- WEIR, B. S. 1990. Intraspecific differentiation. In: Hillis, D. M. and Moritz, G. (eds) *Molecular Systematics*, pp. 373-410. Sinauer Associates Inc., Sunderland, MA.
- WEIR, B. S. AND COCKERHAM, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.
- WOOLHOUSE, M. E. J. 1988. Passive dispersal of *Bulinus globosus*. *Ann. Trop. Med. Hyg.*, **82**, 315-317.
- WOOLHOUSE, M. E. J. AND CHANDIWANA, S. K. 1989. Spatial and temporal heterogeneity in the population dynamics of *Bulinus globosus* and *Biomphalaria pfeifferi* and in the epidemiology of their infection with schistosomes. *Parasitology*, **98**, 21-34.
- WRIGHT, C. A. AND ROLLINSON, D. 1979. Analysis of enzymes in the *Bulinus africanus* group (Mollusca: Planorbidae) by isoelectric focusing. *J. Nat. Hist.*, **15**, 873-885.
- WRIGHT, S. 1978. *Evolution and the Genetics of Populations*, vol. 4, *Variability Within and Among Natural Populations*. University of Chicago Press, Chicago.