Population genetics and dynamics at short spatial scale in *Bulinus truncatus*, the intermediate host of *Schistosoma haematobium*, in Morocco

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SUMMARY

The population biology of the schistosome–vector snail *Bulinus truncatus* was studied in an irrigation system near Marrakech, Morocco using both genetic and demographic approaches. The population genetic survey was conducted in 4 sites, 2 sites being sampled on 2 separate occasions. Individuals were genotyped at 6 microsatellite loci. No variability was found at 4 loci, and the 2 other loci had less than 4 alleles. The differentiation, both spatial and temporal, among populations was extremely weak. The demographic survey was conducted using 2 capture-mark-recapture analyses in 2 separate sites, the first in 1999 and the second in 2000. The second analysis permitted the estimation of parameters based on recent methodological developments (multisite models). Although these studies provided information on several traits, we report here on dispersal only. Both analyses showed that individual dispersal is of the order of a few hundreds of metres per reproductive life, that is the scale of the whole irrigation area. Both the genetic and demographic studies indicated that this area harbours a single – or no more than a few – populations of *B. truncatus*. This has implications for our understanding of the coevolutionary process between snails and flukes.

Key words: microsatellite, freshwater snail, selfing, population genetics, population dynamics.

INTRODUCTION

Recent models of the coevolutionary process showed that population biology of the interaction between hosts and parasites is best understood when examined at the metapopulation level (Gandon *et al.* 1996; Thompson, 1999). In these models, coevolution is perceived as a dynamic interaction between local adaptation within populations and migration among populations. It is, therefore, important to determine empirically both the limits of populations and the rate of migration, for both hosts and parasites. This information could be obtained, by studying both population dynamics (references in Clobert *et al.* 2001) and population genetics (Slatkin, 1985; Rousset, 2001).

We focus here on the population biology of pulmonate snails that serve as intermediate hosts of schistosomes, which has received considerable attention in an effort to collect useful information for disease control (see e.g. Brown, 1994). The population dynamics of these snails have been studied by classical approaches, based on the temporal census of cohorts (e.g. O'Keeffe, 1985; Loreau & Baluku, 1987). The potential of studies based on capture-mark-recapture (CMR) techniques (Lebreton *et al.* 1992; Lebreton, Pradel & Clobert, 1993; Schwarz & Seber, 1999) has remained unappreciated, notwithstanding they provide unbiased estimates of several demographic parameters such as probabilities of survival. An exception is the work of Woolhouse (1988*a*, *b*) and Woolhouse & Chandiwana (1990) who essentially used these techniques for estimating local population size, but also obtained some estimates of dispersal. Further, the demographic studies in snails were essentially concerned with estimating parameters at the population level. Recent methodological developments, however, allow an analysis of processes among populations, such as dispersal (Ims & Yoccoz, 1997; Nichols & Kaiser, 1999). The population genetics of snail hosts has been studied in several species (e.g. Viard, Justy & Jarne, 1997*b*; Webster *et al.* 2001; Mavarez *et al.* 2002; Charbonnel *et al.* 2002; Mavarez *et al.* 2002; reviewed by Jarne & Théron, 2001), and has benefited from recent technical and methodological innovations. This has improved determination of population genetic parameters, such as gene flow (Rousset, 2001). For example, the power of statistical analyses has substantially increased when using hypervariable markers (Jarne & Lagoda, 1996; Estoup & Angers, 1998). However, studies combining CMR and

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genetic data are scarce, and none has been conducted in host snails to our knowledge.

The goal of this paper is to analyse populations of *Bulinus truncatus* on a limited spatial scale, from both demographical and genetical perspectives. More precisely, we were interested in evaluating whether a set of populations under study constituted a single population or a metapopulation. Our study was conducted in an irrigation system near Marrakech, Morocco, that is at a spatial scale of a few kilometers. Four sites were sampled (2 of which were sampled on 2 separate occasions), and individual snails were typed at 6 microsatellite markers in order to conduct genetic analyses. Two CMR experiments were conducted in 2 different sites (using slightly different protocols) on the same irrigation system. These experiments permitted estimation of several parameters of population dynamics, such as capture probability or survival. Here we describe dispersal data in relation to population limits and structure.

MATERIALS AND METHODS

The species studied and sampling area

Our study focused on the freshwater snail *B. truncatus* (Gastropoda, Pulmonata) which is distributed over most of Africa, several Mediterranean islands and part of the Middle East. Three main aspects of its biology are relevant to the current study. (i) Its habitats are usually submitted to annual variation in water availability, which causes wide fluctuations in snail density (Brown, 1994; Madsen *et al*. 1988) and has a marked influence on the distribution of genetic variability (Viard *et al*. 1997*b*). Density fluctuations are expected to be more limited in the artificial habitat analysed here. (ii) *B. truncatus* is an hermaphrodite in which the selfing rate is generally high (Doums *et al*. 1996; Viard, Doums & Jarne, 1997*a*). It also displays a peculiar sexual polymorphism called aphally which markedly influences the selfing rate (reviewed by Doums, Viard & Jarne, 1998*b*). (iii) *B. truncatus* is one of the major vectors of several species of *Schistosoma*, the agents of bilharziases in humans and cattle in Africa (Brown, 1994). It serves as the intermediate host of *Schistosoma haematobium* in Morocco (Brown, 1994; Laamrani *et al*. 2000), and urinary bilharziasis is still detected in the area studied, though at a very low prevalence (G. Chlyeh, unpublished results).

The whole study was conducted within the irrigation system of Oulad Sid'cheikh, 20 km South of Marrakech. A schematized map of this system is presented in Fig. 1. Ground water is pumped to fields and orchards through a series of primary and secondary concrete canals. Pairs of sinks, 0·5 to 2 m deep, are located about every 100 m along these canals. Sinks of a pair are separated by about 10 m. As water may run as fast as 0·4 m/s in the canals, water turbulence within the first sink of a pair is much higher than in the second. This mostly limits the populations of *B. truncatus* to the second sink (settled populations of *B. truncatus* were never found in the canals). As water is pumped at several pumping stations, some of the sites studied (see below) are directly connected by running water, allowing for potential dispersal following water currents. Not all sites are connected (Fig. 1). A consequence of water current is that dispersal between pairs of sinks occurs downstream.

Population genetic analysis

Four sites were sampled in Oulad Sid'cheikh. Two of them were sampled twice at a 1-year interval (Table 1 and Fig. 1). Note that sites 1P2, 2P1 and 3P2 are directly connected by water current, and are separated from site 4P1. Once collected, snails were preserved in 95% ethanol until DNA extraction. DNA was extracted from whole snails using the QIAamp DNA Mini Kit (Qiagen). The microsatellite loci analysed are those described by Jarne (1994), 3 of which have already been used in *B. truncatus* populations from Morocco, including one form Oulad Sid'cheikh (Sidi Chick in Viard *et al*. 1997*b*). Technical details about these loci are given in Table 2. Amplification reactions were carried out separately for each locus in 12 μl volumes including 1 μl of 10 μM fluorescent-labelled primer (Table 2), 1·2 μl of 10 × reaction buffer, 1·2 μl of 25 mM $MgCl_2$, 0·3 μl of 20 mM dNTP mix, 0·05 μl of 5 U/μl *Taq* DNA polymerase and 2 μl of a 1/50 dilution of genomic DNA. PCR was conducted using a PTC100 thermocycler (MJ Research) under the following conditions: 40 sec of denaturation at 94 °C, and 40 cycles of 10 sec of denaturation at 92 °C, 15 sec of annealing at 50–59 °C (Table 2) and 30 sec of extension at 70 °C. PCR products were analysed using an ABI Prism 310 Genetic Analyser, and primers were labelled to allow multiplexed runs. PCR products (1 μl per locus) were pooled in 20 μl of deionized formamide and 0·4 μl of GeneScan-500ROX Size Standard.

Genetic variation within populations was described using several standard parameters. We estimated the number of alleles, the observed heterozygosity and gene diversity (Nei, 1987) per locus and population. Departures from Hardy–Weinberg equilibrium at each locus were tested within each population using exact tests (Rousset & Raymond, 1995). One locus only turned out to be polymorphic enough to allow for this test (Bt13). Departure over all populations was evaluated using Fisher's method. The unbiased estimator $\hat{f}$ of Wright's inbreeding coefficient F_{is} was calculated according to Weir & Cockerham (1984). We also estimated the selfing rate using the relationship $\hat{s} = 2\hat{f}/(1+\hat{f})$ (see Rousset,

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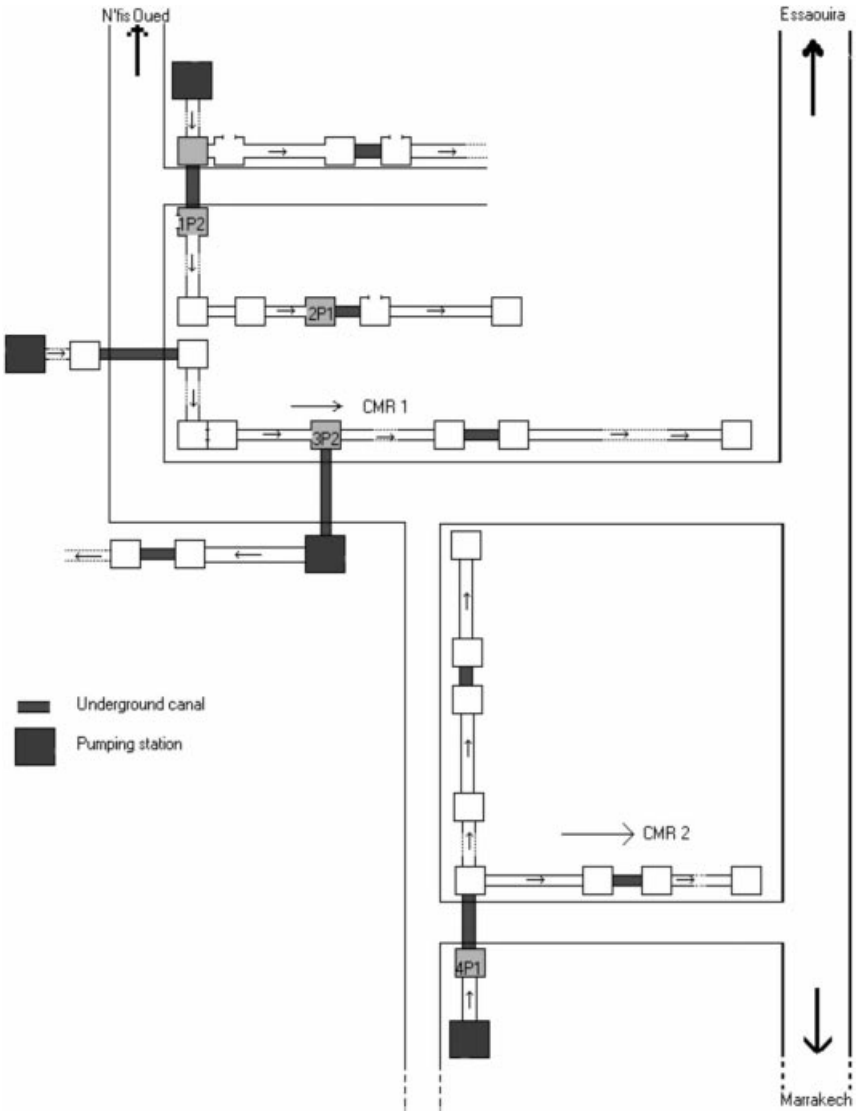

Fig. 1. Schematized map of the Oulad Sid'cheikh irrigation scheme, located along the Marrakech–Essaouira road, giving the location of sampled sites (1P2, 2P1, 3P2 and 4P1; empty squares represent sinks), their connections through half-pipes (underground canals are darkened) and the movements of water (arrows within pipes). Pairs of sinks (e.g. downstream to 3P2) are located about every 100 m along these canals. Sinks of a pair are separated by about 10 m. The location of the CMR experiments are indicated by CMR1 (1999) and CMR2 (2000). Filled squares are pumping stations. Note that the map is not to scale, and the largest distance along the Marrakech–Essaouira axis is 3 km.

Table 1. Names of sampled sites, sampling date and number of individuals sampled (*N*)

(See Fig. 1 for their location.)

Sample	Sampling date	*N*
1P2	Nov. 1998	20
	Nov. 1999	10
2P1	Nov. 1998	20
	Nov. 1999	20
3P2	Nov. 1999	20
4P1	Nov. 1998	15

1996; Viard *et al*. 1997*a*). Genetic differentiation was analysed per locus both over all pairs of populations and all populations using a homogeneity test (Goudet *et al*. 1996) computed as an exact test. The estimator $\hat{\theta}$ of F_{st} (Weir & Cockerham, 1984) was calculated per locus and over all loci, both for each pair of populations and over all populations. Calculations and tests were performed using GENEPOP 3.1c (Raymond & Rousset, 1995).

Population dynamics analysis

The dynamics of *B. truncatus* populations were analysed using CMR methods (Lebreton *et al*. 1992) in 2 separate surveys. As mentioned in the Introduction section, we focus on those results pertaining to dispersal, that is movements between sinks. CMR studies were conducted in 2 successive years in 2 different sites (Fig. 1). Snail sampling in sinks was

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Table 2. Core sequences of the microsatellite loci used with their primer sequences (5′ to 3′) and annealing temperature (T; a range refers to touchdown PCR)

(The last column gives the number and size of alleles detected over all sites given in Table 1. 0 at locus 12 refers to a null allele. See also Jarne *et al*. (1994) and Viard *et al*. (1997*c*).)

Locus	Core sequence	Primers	T	Size
Bt1	$(CA)_{12}(GA)_4$	CGTGGGGACTGTTTACTTTAC	50	1
		CCCCCTAAAAGTTTGGTCTAG		186
Bt4	$(TC)_{31}$	CAATCTTGTATCTATAATCCG	57	1
		CCACTCCAGTAAGAAACAAAC		139
Bt5	$(CA)_{22}$	CCTGTTTTCTTCTGAATATCTT	50–55	1
		CTCGTATTGGTCTCCATGTT		147
Bt12	$(GATA)_{36}$	TGAAACATGTTTTACGCATTG	50–55	3
		ACATACGGCTAACAATTTGTATTAC		0; 239; 243
Bt13	$(GATA)_{33}$	CACAAGATGGACAGGTACCACATGG	55–59	4
		CAAGTTTACAATTGCCTTGCATTTC		307; 315; 319; 323
Bt23	$(CT)_{22}$	TCATCCAGTGTAGGTTTCAGTCT	50	1
		CTATTGAGCACCCACCGGGAG		229

performed using a metallic dredge (Khallaayoune & Laamrani, 1992), which sampled individuals larger than 5 mm in shell length.

(i) The first experiment was conducted in winter 1999 in a series of sinks located downstream of sink 3P2 (Fig. 1). *B. truncatus* individuals from sink S1 (the first sink downstream to 3P2) were sampled exhaustively on 3 February 1999, measured (shell length from apex to last spire), marked using coloured nail varnish, and released in S1. Recaptures were performed every 2 days on 3 occasions (5–9 February): all snails captured were again measured, marked (1 varnish colour per marking occasion), and released in S1. A last recapture was performed on 9 March 1999. On the 4 recapture occasions, we also looked for marked *B. truncatus* individuals in sinks S0 upstream, and S2 to S9 (sinks were numbered consecutively) downstream in order to document snail movements among sinks.

(ii) The second experiment was conducted in spring 2000 in a series of sinks located downstream of sink 4P1 (S′0 to S′11, with S′0 the first sink downstream to 4P1; Fig. 1), following a slightly different protocol, which allowed for multisite CMR analyses. Two sessions of capture (3 occasions per session, separated by 3 days) were separated by a period of 19 days. On April 21, sinks S′1, S′3, S′5 and S′7 were exhaustively sampled using the same dredge as above. All captured individuals were measured, marked using numbered, coloured plastic tags, and released. The same process was followed on 3 successive sessions (24 April to 16 May). Unfortunately, the last 2 recapture sessions (20 and 24 May) resulted in no recapture of living individuals, because of molluscicide treatment by the Marrakech SIAAP (Service d'Infrastructure et d'Action Ambulatoire Préfectoral) on 18 May. Marked individuals were also searched for in sinks S′9 and S′11 in all sessions. A preliminary survey indicated that even-numbered sinks (S′0 to S′10) harboured very low number of snails, and they are not considered here.

Movement data from the first experiment cannot be analysed using the formal framework used in multisite CMR studies because snails were marked in a single sink (S1), which prevents controlling for site- and time-dependency of recapture probability. Moreover, results from both experiments showed that temporal and spatial variation in the probability of recapture and survival cannot be neglected (G. Chlyeh & P.-Y. Henry, unpublished results). As a consequence, it was not possible to get unbiased estimates of dispersal. However, this experiment did provide information on the distribution of dispersal distance (e.g. the average distance) which can be compared to values from the second experiment. Dispersal in the second experiment was analysed using the methodology developed for multisite CMR designs (Hestbeck, Nichols & Malecki, 1991). Variation in recapture probability with time and among sites was taken into account when estimating transition probabilities between sites. However, as very few movements were documented (20 out of 837 marked individuals), some sources of variation in movement probability (e.g. time- or sink-dependence) could not be explored. A single multisite model was considered. Survival and recapture probabilities were modelled according to the results from a local survival analysis (G. Chlyeh & P.-Y. Henry, unpublished results). Movement was characterized by the probability of moving from one sink to the sink downstream between pairs of successive recapture occasions. As upstream movement was not documented, the relevant probability was assumed to be null. As the sample size was quite small in S′1, only data from S′3, S′5 and S′7 were considered in the analysis.

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Table 3. Observed heterozygosity (H_o), gene diversity (H_e), estimate of F_{is} (f) and the probability (P) of the associated exact test on Hardy–Weinberg equilibrium, and estimate of the selfing rate (S) for the 4 sites studied

(Time refers to the sampling date (t_1 and t_2 holds for November 1998 and November 1999 respectively). Note that f (and therefore S) and p are given for locus Bt13 only, because of monomorphism at other loci.)

Pop.	Time	H_o	H_e	f	P	S
1P2	t_1	0·017	0·055	0·709	0·002	0·830
1P2	t_2	0·017	0·044	0·654	$< 10^{-4}$	0·791
2P1	t_1	0·017	0·055	0·709	0·002	0·830
2P1	t_2	0·017	0·078	0·772	0·001	0·871
3P2	t_2	0·000	0·042	1·000	3×10^{-4}	1·000
4P1	t_2	0·011	0·031	0·659	0·034	0·794

Table 4. Number of individuals that were marked and released in S1, and then recaptured in downstream sinks (from February 3 to February 9), as a function of the recapture date (Feb. holds for February) in the first experiment (1999)

(The value in parentheses is the total number of snails captured.)

Sink	Feb. 5	Feb. 7	Feb. 9	March 9
S2	2 (14)	0 (16)	0 (9)	0 (0)
S3	0 (316)	2 (214)	5 (322)	21 (210)
S4	0 (143)	0 (26)	0 (31)	0 (0)
S5	0 (512)	0 (413)	11 (317)	0 (265)
S6	0 (45)	0 (25)	0 (14)	0 (12)
S7	0 (422)	0 (325)	1 (217)	0 (157)
S8	0 (33)	0 (27)	0 (18)	0 (8)
S9	0 (425)	0 (311)	0 (282)	0 (243)

Table 5. Matrix of movements among sinks documented by CMR in the second experiment (2000), pooled over all recapture occasions.

(The matrix gives the number of individuals having moved from sink i to sink j. Note that movements occurred downstream only. The values in parentheses are the total numbers of snails marked (first line) and captured (first column) per sink respectively.)

Sink i	S′1 (51)	S′3 (503)	S′5 (125)	S′7 (158)
Sink j				
S′3 (290)	1		–	–
S′5 (55)	1	9		–
S′7 (100)	2	2	4	
S′9 (1)	0	0	0	1
S′11 (0)	0	0	0	0

RESULTS

Genetic variability

PCR products were obtained at the 6 loci used in the 6 samples studied, although a null allele was detected at locus 12 (Table 2). Four of them showed no variability, while up to 4 alleles were detected at locus Bt13, when considering all populations. The number of alleles averaged over the sites (sometimes including 2 sampling dates) ranged between 1 at the 4 monomorphic loci and 3 at locus Bt13. The number of alleles per locus and site was never higher than 3. The observed heterozygosity was also very low, and much lower than gene diversity (Table 3). This is consistent with the rejection of the hypothesis of Hardy–Weinberg equilibrium in all samples, as well as with high estimates of the selfing rate (higher than 0·8). However, this should be regarded with caution since a single locus (generally Bt13) showed variation within samples.

The test of population structure per locus and pairs of populations was performed for loci Bt12 and Bt13 only. Note that tests were performed using all 6 samples when differences were detected. No significant difference was detected at locus Bt12 (5 out of 5 possible tests), while 5 tests, out of 15, were highly significant ($P < 10^{-6}$) at locus Bt13. All significant results at Bt13 involved samples 4P1 on one side, and the other populations on the other side. This is reflected on the matrix of pairwise F_{st} estimated over all loci (not shown), values of which were low, except when this population was considered against the others. The exact test of differentiation conducted over all populations was not significant at locus Bt12 ($P = 1$), but was at locus Bt13 ($P < 10^{-6}$).

Demographic results

The raw results of movements in the first experiment are presented in Table 4. No snails were recaptured in S0. Distances between S1 and the sink in which migrants were captured give an estimate of dispersal distance. Fourty-two individuals were recaptured outside S1, out of 1309 marked individuals in S1 and 5371 collected in S2 to S9 over the whole experiment (Table 4). Most were recaptured in S3 (28), and half on the last recapture occasion (9 March). The distance between successive pairs of sinks being approximately 100 m, the average distance for migrants is about 130 m. However, the uneven distribution of migrants among downstream sinks suggest variation in dispersal or recapture probability, and this estimate should be cautiously interpreted.

In the second experiment, 20 individuals were recaptured outside the sink in which they were marked, out of 837 marked individuals (Table 5).

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Nine of these individuals were marked in S′3 and recaptured in S′5. As above, we can estimate the average distance of dispersal for those individuals that moved as about 135 m. As most movements (18) occurred during the 20-day period between the 2 marking sessions, the average distance of dispersal was similar in the 2 experiments. From a local survival analysis (G. Chlyeh & P.-Y. Henry, unpublished results), the best model retained, on the basis of minimum AIC (Akaike information content; Burnham & Anderson, 1998), includes the effects of both sink (s) and time (t), and their interaction, on capture probability P, and additive effects of sink (p) and squared snail size (L^2) on survival probability Φ (model $\Phi(L^2+s)$ P(s*t)). A constant movement probability (m) was therefore estimated from model $\Phi(L^2+s)$ P(s*t) m(.). This produced an estimate of the probability of moving from one sink to the sink downstream over a 3-day period of $0{\cdot}024 \pm 0{\cdot}007$ (95% confidence interval: 0·014–0·042).

DISCUSSION

Genetic variability

Limited variability was detected within sinks, with only 1 polymorphic locus out of 6 loci studied (locus 13 displayed 2 alleles in a single site). Moreover, very few heterozygotes were detected. The variability detected is consistent with what has been previously observed in a single site downstream to 3P2 from the same irrigation system (Viard *et al*. 1997*b*). These authors found no variation at the 3 loci they had in common with the present study, and the alleles detected are those with highest frequency in 3P2. On the other hand, the 3 loci showed ample variation in other populations of *B. truncatus*, with up to 10 alleles per loci in Niger populations (Viard *et al*. 1997*b*, *c*). Poorly variable markers is therefore not a likely explanation to the pattern observed in our study. At least 2 alternative explanations can though be proposed (review in Städler & Jarne, 1997). First, *B. truncatus* is a highly selfing species, as shown by the large-scale study of Viard *et al*. (1997*a*, *b*). Our previous study has shown that the Moroccan populations are no exception (Viard *et al*. 1997*b*). Moreover, the ratio of aphallic individuals is extremely high in the site studied here (over 0·8; G. Chlyeh, unpublished data) which certainly leads to even higher selfing rates (Doums *et al*. 1998*b*). Self-fertilization is known to depress the fraction of heterozygous genotypes, but also to depress variability because it enhances the action of genetic drift and indirect selection (Charlesworth, Morgan & Charlesworth, 1993). Secondly, the local dynamics may impose genetic drift on populations and limit the amount of variation. We mentioned above that populations of freshwater snails in Africa experience cycles of drought/flood that markedly affect the density of populations (Brown, 1994). Although such cycles are not observed in the system studied here, our own temporal surveys in 11 sinks, including the 4 studied here, suggest that populations are regularly going through low density (of individuals larger than about 4–5 mm), although it is hard to relate this variation to environmental factors. A second possibility is related to the history of the irrigation system which was built about 30 years ago. Quite likely, the number of founding individuals of *B. truncatus* populations was limited, and of local origin (see Viard *et al*. 1997*c*). In other words, populations experienced a founding event. Recurrent treatments of the irrigation system using molluscicides may be a further cause of population bottlenecks, since their application in severe depression of populations (as was unfortunately experienced in the second demographic study). Their influence should be mitigated, because molluscicides are not applied to the whole irrigation system at once. Even in those areas in which molluscicides are applied, underground canals (between pairs of sinks; see Materials and Methods section) constitute refuges.

A second noteworthy aspect of our results is the homogeneity observed among populations. Sites 1P2, 2P1 and 3P2 were not different, and can be considered as a single population. This is confirmed by the temporal analysis in 1P2 and 2P1 which also showed no variation. Site 4P1 turned out to be significantly differentiated at the only polymorphic locus (Bt13). This was not completely unexpected, since 4P1 is separated from other sinks. A tentative conclusion would be that the irrigation system of Oulad Sid'cheikh harbors at least 2 populations, though not much more. However, we should be cautious here, because Bt13 is likely to be an extremely mutable locus (Viard *et al*. 1997*b*). Mutation could then out compete migration in the production of within-population variability, and the pattern observed would result more from mutation than from limited migration. At the other loci, mutation would not be high enough to introduce variation.

These results can be compared to other studies conducted at similar or slightly larger geographical scale in populations of tropical snails acting as intermediate hosts for schistosomes (*Bulinus* and *Biomphalaria*). Note first that more genetic variation has been detected in these studies, whether they were based on RAPDS (Sire *et al*. 2001; Webster *et al*. 2001) or on microsatellites (Viard *et al*. 1997*b*, *c*; Charbonnel *et al*. 2002; Mavarez *et al*. 2002). An interesting result of all these studies is that significant genetic differentiation may occur even among populations separated by several hundreds of metres, as has been found here between 4P1 and the other populations. However, differentiation might be more readily observed in temporarily unstable habitats than in permanent habitats, as observed by Webster

et al. (2001) in *Biomphalaria pfeifferi*. That no differentiation was observed between 1P2, 2P1 and 3P2 is consistent with this generalization, since the local populations are connected by continuous water currents. However, Viard *et al*. (1997*c*) observed more differentiation in permanent than in temporary habitats in Niger populations of *B. truncatus*. It is also likely that differentiation fluctuates in time with the alternance of dry and rainy seasons, as shown in Malagasy populations of *B. pfeifferi* (Charbonnel, 2001). The environmental conditions experienced by snail populations in the irrigation system studied here are probably too buffered, since water comes from the ground, to allow for such variation.

Demographic results

The 2 CMR experiments indicated that individuals of *B. truncatus* are highly mobile. This can be viewed from 2 perspectives. (i) Movements of up to 300 m were documented in both experiments over the course of about 3 weeks. The average distances were 130 and 135 m in Exps 1 and 2 respectively. This provides a conservative estimate of dispersal distance for those individuals, which have moved and survived, of about 100 m per month. (ii) Exp. 2 was designed such as to take full advantage of multisite CMR analyses, and provided the probability of snail movement over 3-day periods between neighbouring sinks (separated by 100 m). Assuming a reproductive life-expectancy of 150 days (from the laboratory data of Doums *et al*. 1998*a*) and a binomial distribution of movement (moving versus non-moving individuals), the probability that an individual spends its entire reproductive life in a sink is only 0·3. In other words, about 70% of individuals will move, and probably reproduce in the same sink different from the one in which they grew up. Under the same assumptions, the probability of moving 400 m downstream is 0·025. The values obtained are consistent with the fact that fast running water in the irrigation system studied may promote dispersion. However, the absolute distance should be considered with caution, since they are strongly related to the distance between sinks.

CMR methods have rarely been used in freshwater invertebrates, and even less in those molluscs species transmitting trematode parasites, such as schistosomes. The few estimates of passive dispersal distance, based on smaller groups of marked individuals, were reported by Woolhouse (1988*b*). Although the studies were in natural habitats (rivers), the order of magnitude obtained is similar to the one given by our study, that is in the order of hundreds of metres over a life-cycle. This comparison should be considered with caution, since dispersal distances in our study strongly depend on the distance between sinks. Woolhouse (1988*b*) also cautioned that these values seriously depend on local hydrology, itself partly depending on rains. This holds in the irrigation system studied, and may also be applied to populations in more temperate conditions. Given that the fraction of emigrating individuals at that temporal scale is quite high (see above and Woolhouse, 1988*b*), hundreds of metres (at the square) can be retained as a conservative estimate for population area.

Population or metapopulation?

The genetic analysis suggests that the sites studied correspond to 2 populations. This is consistent with the CMR studies from which it can be inferred that sinks freely exchange migrants. The probability that an individual migrates from one sink to the next sink downstream is indeed 70%. Note that the 2 CMR experiments were conducted in 2 parts of the irrigation system in which populations are genetically differentiated (the 1st experiment was conducted in the part associated with 1P2, 2P1 and 3P1, while the second was conducted in the part associated with 4P1), and led to similar results on movement distance. Although the genetic variability was limited at the system scale, we can tentatively infer that there are at least 2 populations which may be part of a metapopulation at a larger geographical scale. Similar results have been obtained in very different environmental conditions in Niger populations of *B. truncatus* (Viard *et al*. 1997*c*).

What are the conclusions in relation to parasites? Our results suggest that the irrigation system studied corresponds at best to a few populations. First, our study may be helpful in a practical perspective (see Laamrani *et al*. 2000). Chemical control of snails, which has been performed by the SIAAP over the last 30 years, has not been extremely efficient in actually controlling snail populations. Our demographic results suggest that this is likely to be due to significant movements between sinks and therefore extremely fast recolonization. Treatments should therefore be performed at population scale, that is including all sinks. Moreover, even in those areas in which molluscicide is applied, underground canals (between pairs of sinks; see Materials and Methods section) constitute refuges. Secondly, the outcomes of the coevolutionary process between hosts and parasites depend on population structure, and a critical aspect is our capacity to determine the limits of populations, that is the scale at which local adaptation can occur (Gandon *et al*. 1996; Thompson, 1999; Jarne & Théron, 2001; Webster & Darvies, 2001). Our results suggest that the irrigation system studied corresponds at best to a few populations. Of course, it remains to be determined whether these populations are connected to other populations, occupying natural or artificial habitats.

Moreover, no larval forms of parasitic trematodes have been detected in this irrigation system over a monthly 2-year study involving 11 sinks (G. Chlyeh, unpublished data). The most obvious reason is that parasitic cycles cannot be completed, because of the absence of final hosts when animals act as final host or of human behaviour when humans are final hosts. It is, therefore, not possible to define parasitic populations in this irrigation system. However, this should be possible in principle, using genetic markers (see examples in Jarne & Théron, 2001). Future studies on fluke/snail coevolution should certainly be aimed at analysing population structure, in addition to studies on the interaction between flukes and snails, if a clearer view on the coevolutionary process has to emerge (Jarne & Théron, 2001; Webster & Davies, 2001).

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