DISPERSAL IN A PARASITIC WORM AND ITS TWO HOSTS: CONSEQUENCE FOR LOCAL ADAPTATION

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Abstract.—Characterizing host and parasite population genetic structure and estimating gene flow among populations is essential for understanding coevolutionary interactions between hosts and parasites. We examined the population genetic structure of the trematode *Schistosoma mansoni* and its two host species (the definitive host *Rattus rattus* and the intermediate host *Biomphalaria glabrata*) using microsatellite markers. Parasites were sampled from rats. The study was conducted in five sites of the Guadeloupe Island, Lesser Antilles. Mollusks display a pattern of isolation by distance whereas such a pattern is not found neither in schistosomes nor in rats. The comparison of the distribution of genetic variability in *S. mansoni* and its two host species strongly suggests that migration of parasites is principally determined by that of the vertebrate host in the marshy focus of Guadeloupe. However, the comparison between genetic differentiation values in schistosomes and rats suggests that the efficacy of the schistosome rat-mediated dispersal between transmission sites is lower than expected given the prevalence, parasitic load and migration rate of rats among sites. This could notably suggest that rat migration rate could be negatively correlated to the age or the infection status of individuals. Models made about the evolution of local adaptation in function of the dispersal rates of hosts and parasites suggest that rats and mollusks should be locally adapted to their parasites.

Key words.—*Biomphalaria glabrata*, gene flow, local adaptation, multihost parasite, population genetic structure, *Rattus rattus*, *Schistosoma mansoni*.

In host-parasite systems, each species constitutes an ever-changing environment to which its opponent has to adapt. In such a variable environment, adaptation depends on both the nature and the strength of selection (Lively 1999), but also on the evolutionary potential of interacting species, that is their ability to incorporate genotypes able to overcome the weaponry put forward by the opponent (Gandon and Michalakis 2002). Several theoretical studies have now investigated the role of various demographic factors affecting the coevolutionary outcome of host-parasite interactions in a metapopulation (e.g., extinction and migration; Gandon et al. 1996; Thrall and Burdon 1997; Kaltz and Shykoff 1998). Gene flow among populations plays a prominent role determining the spread of both resistance (in hosts) and virulence (in parasites) genes (Gandon et al. 1996), and the relative parasite and host dispersal rates among populations strongly affect the evolution of local adaptation (Gandon et al. 1996; Lively 1999; Gandon and Michalakis 2002). For example, under a matching-allele model of infectivity, at low to intermediate migration rates, parasites (hosts) are locally adapted when they migrate more than hosts (parasites). This seems counterintuitive because gene flow is generally expected to weaken local adaptation (Lenormand 2002). However, in an environment changing through time, as under a cyclical co-evolution model, factors that increase the evolutionary potential of species by incorporating novel alleles within subpopulations also increase their ability to respond to these changes (Gandon and Michalakis 1996).

Given the importance of gene flow and demographic patterns on local adaptation, it has been suggested that studying the genetic structure of populations in both hosts and parasites is a prerequisite for understanding the evolution of pathosystems (Thompson 1994; Dybdahl and Lively 1996; Jarne and Théron 2001). This has been done in a limited number of studies (e.g., Mulvey et al. 1991; Dybdahl and Lively 1996; Delmotte et al. 1999; Davies et al. 1999; Mutikainen and Koskela 2002), and it remains unclear whether gene flow is more intense in hosts or in parasites. Moreover, some parasites exhibit complex life cycles, meaning that they sequentially exploit different hosts during their life span (the adult part of the life cycle is by definition spent in the definitive host; other hosts are referred to as intermediate): no study has investigated the population genetic structure of both the parasite and its host species. For example, Mulvey et al. (1991) restricted their analysis to a parasite (*Fascioloides magna*) and its definitive host (white-tailed deer), whereas Dybdahl and Lively (1996) focused on a parasite and its intermediate host, leaving aside in both cases at least one important host. Several reasons can be invoked for studying the population genetic structure of all host species and of the parasite in such a situation: (1) Each host species constitutes a specific ever-changing antagonistic environment to which the parasite has to adapt. The spatial heterogeneity of the host-environment system partly depends on population genetic structure. (2) The machinery required for infection, exploitation, and transmission is likely to vary from host to host, and hence the selective pressures acting on parasites in different host species. (3) Such a variation is also expected for life-history and demographic traits (e.g., generation time, dispersal rate, effective population size) with a correlated

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variation in evolutionary potential between the species involved in the coevolution. The evolutionary trajectory of parasites can be especially disturbed in case of a trade-off in infectivity/virulence between the different hosts (e.g., Davies et al. 2001). Coevolution and local adaptation in multihost parasites may differ from those in direct life-cycle parasite.

We here focus on *Schistosoma mansoni*, a parasite with two hosts which exhibit marked differences in life-history traits (notably dispersal abilities) and presumably population structure. *Schistosoma mansoni* is a dioecious blood trematode (Combes 2001). In Guadeloupe (French West Indies), its cycle relies on the black rat *Rattus rattus* (definitive host) and the freshwater snail *Biomphalaria glabrata* (intermediate host) (Théron and Pointier 1995). Transmission occurs along the marshy forest of Grande Terre Island (Fig. 1) which experiences seasonal flooding (August to December) and drought (April to July). Eight transmission sites separated by about one to 10 kilometers have been reported (Théron and Pointier 1995). The prevalence of *S. mansoni* in the intermediate host is generally very low (0.2–4%) and infections are generally monomiracidial (Sire et al. 1999). Mollusk population size and abundance within transmission sites varied with water level, the highest size and density being observed during the rainy season (Sire et al 1999). For example, up to 3000 mollusks per hectare have been found in Dans Fond in December 1995 (rainy season), whereas the snail population was reduced to seven residual puddles in June 1997 (dry season). Moreover, the freshwater habitats are poorly connected, even during the rainy season. We therefore expect limited dispersal and isolation by distance in snails (see Langand et al. 1999; Mavarez et al. 2002). On the other hand, the prevalence is high in rats (up to 100%; Théron and Pointier 1995) and the parasitic load can reach up to 1000 worms per rat (Sire et al. 2001a). Rat density reaches about 14 individuals per hectare in the marshy forest (Delattre and Le Louarn 1981). Rats exhibit marked territoriality and limited movements within transmission sites (Delattre and Le Louarn 1981). No direct information is currently available on their mobility among transmission sites, notably on long-distance dispersal (but see Sire et al. 2001b). This is unfortunate since dispersal in *S. mansoni* is certainly mediated by rats, given the high parasite prevalence and abundance in rats.

On the whole, migration is expected to differ markedly between mollusks and rats: mollusks are expected to disperse at short distance only and to display a pattern of isolation by distance over transmission sites, whereas rats are expected to disperse at much larger distance with no relation between geographic and genetic distance at the scale studied. As mentioned above, dispersal in schistosomes among transmission sites is expected to be mainly mediated by rats. We therefore expect similar patterns of dispersal between rats and schistosomes at this scale. However, it is much more difficult to predict the relative dispersal rates of host and parasite species, which are required for predicting patterns of local adaptation.

The aim of this article is to study the population genetic structure of *S. mansoni* and its two hosts (*R. rattus* and *B. glabrata*) in the same sites from the marshy forest focus of Guadeloupe using microsatellite markers and therefore evaluate dispersal patterns in these three interacting species. Our
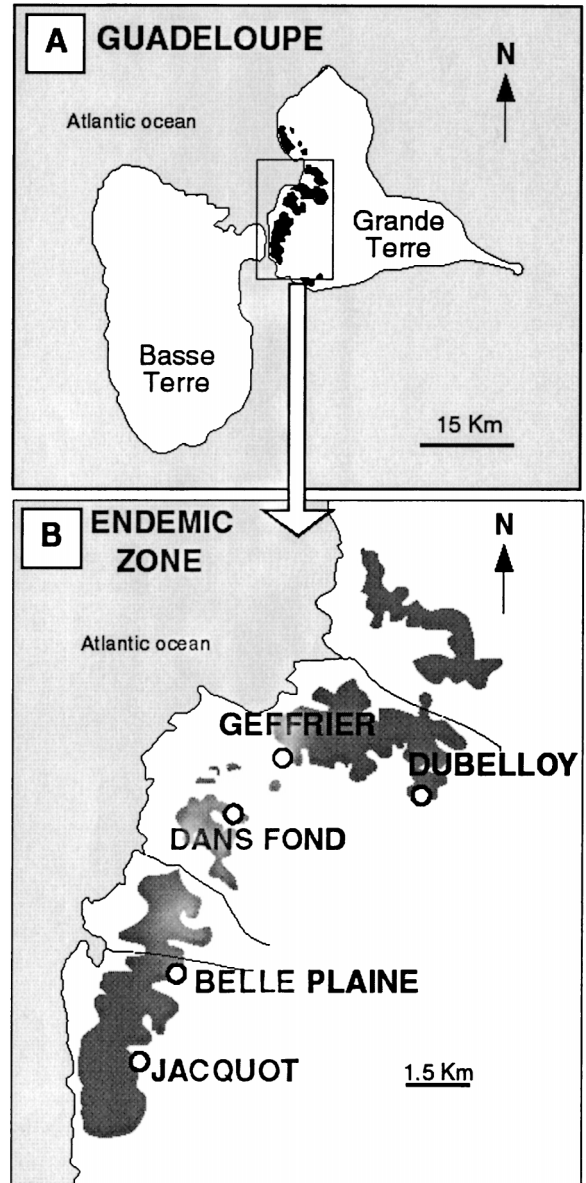

FIG. 1. Location of the five transmission sites in the marshy forest focus of Guadeloupe where sampling of *Schistosoma mansoni*, *Rattus rattus*, and *Biomphalaria glabrata* was performed. (A) Location of the marshy forest in Guadeloupe. (B) Location of the transmission sites: Jacquot, Belle Plaine, Dans Fond, Geffrier, Dubelloy.

results are discussed in relation to the implementation of local adaptation in a multihost/parasite system.

MATERIALS AND METHODS

Parasite Life Cycle

In the definitive host, sexually mature worms infect the mesenteric venules and pass eggs into freshwaters via host feces. A single miracidium (the first free-living motile larval stage) hatches per egg and penetrates the intermediate host in which a period of asexual reproduction occurs prior to cercarial development. Cercariae (the second free living motile larval stage) leave the snail and infect the definitive host.

TABLE 1. Ecological and genetical characteristics of host and parasite samples collected from the five transmission sites of the marshy forest focus of Guadeloupe (JAC, BLP, DFO, GEF, DUB). *Hs* refers to the genetic diversity (Nei 1987), *f* to the estimate of F_{IS} within subpopulations (infrapopulations for schistosomes) and θ to the estimate of F_{ST} between infrapopulations of parasites (Weir and Cockerham 1984). *N* is the number of individuals studied, *SR* the sex ratio (in rats), *P* the prevalence (% of infected rats), *I* the intensity of infection (number of parasites per infected rat ± standard error). For *Schistosoma mansoni*, *Nt* refers to the total number of schistosomes found within all rats in each site. Snail data from the two DFO sites were pooled. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant at the 5% level.

	JAC	BLP	DFO	GEF	DUB
Rattus rattus					
N	25	24	22	33	27
SR	0.72	0.58	0.5	0.39	0.52
Hs	0.62	0.51	0.62	0.62	0.64
f	−0.021 ns	−0.045 ns	0.003 ns	−0.043 ns	−0.026 ns
Schistosoma mansoni					
Nt	781	845	857	261	456
P	0.96	0.66	0.95	0.39	0.82
I	32.5 ± 6.3	52.8 ± 16	40.8 ± 12.9	18.6 ± 5.4	20.7 ± 6.9
N	43	40	60	29	47
Hs	0.54	0.43	0.47	0.47	0.51
f	0.021 ns	0.036 ns	0.058 ns	−0.039 ns	−0.069*
θ	0.025*	0.002 ns	0.027**	0.065***	0.029**
Biomphalaria glabrata					
N	50	30	75	46	30
Hs	0.73	0.61	0.67	0.52	0.61
f	0.008 ns	0.039 ns	0.036 ns	−0.078 ns	0.076 ns

Sampling

Rattus rattus, *B. glabrata*, and *S. mansoni* were sampled in five sites of the marshy forest focus of Grande Terre Island in Guadeloupe (Fig. 1). The sites sampled included from south to north Jacquot (JAC: N, 16°16.241′; W, 061°31.999′), Belle Plaine (BLP: N, 16°17.405′; W, 061°31.444′), Dans Fond (DFO: N, 16°18.500′; W, 061°30.720′), Geffrier (GEF: N, 16°19.907′; W, 061°29.952′) and Dubelloy (DUB: N, 16°19.660′; W, 061°28.524′). These sites are located along the border of the marshy forest which is fragmented into three main patches of different sizes (Fig. 1). Linear geographic distances among sites vary from about two to 10 kilometers.

Rats were captured in these five sites over five consecutive nights of the dry season (May 2001) using traps baited with coconut (Table 1). Rats were anesthetized following international principles regarding the care and use of vertebrates. A piece of leg muscle was collected from each individual and stored in 70° ethanol. Adult schistosomes were recovered from rats using a standard perfusion technique (Duvall and Dewitt 1967) and they were carefully washed in physiological saline solution. They were counted in order to estimate parasite prevalence (probability of rat infection) and mean parasite load (population size of schistosomes per infected rat).

Because genetic diversity of schistosomes is distributed both within and between definitive hosts within transmission sites in Guadeloupe (see Sire et al. 2001a; Prugnolle et al. 2002), schistosomes coming from four different rats chosen at random from each site were collected and stored in 70° ethanol for genotyping. Within each rat, 20 schistosomes (10 males and 10 females) were collected. Schistosomes sampled from the same rat constitute an infrapopulation.

Mollusks were sampled during the previous wet season (December 2000) to ensure large enough sample sizes. Note that two sites separated by about 200 m were sampled in Dans Fond (DFO₁ and DFO₂) corresponding to two subterranean springs. This allowed evaluating the occurrence of population genetic structure at a very small spatial scale. All mollusks were subsequently stored in 70° alcohol.

Genotyping

DNA was extracted from 387 worms using the method of Durand et al. (2000). Seven microsatellite loci were genotyped (Genbank accession number: AF202965, AF202966, R95529, L46951, M85305, AF325695, AF325697). The characterization of variability was performed following Durand et al. (2000) for loci AF202965, AF202966, R95529, L46951, and M85305 and Curtis et al. (2001) for loci AF325695 and AF325697. Rat DNA was extracted from leg muscle using the QIAmp tissue kit (Qiagen, Valencia, CA). Seven microsatellite loci originally cloned in *Rattus norvegicus* (D5rat83, D8rat28, D9rat13, D10rat20, D11rat56, D16rat81, and D18rat75: Rat MapPairs in the rat screening set, Research Genetics, Inc., at: http://mp.invitrogen.com/mappairs_rat.php3) were genotyped. These microsatellites were adapted by J.-F. Cosson and F. Paquier for *R. rattus* (Cosson and Paquier, unpubl. ms.). Mollusk DNA was extracted from individual foot using the QIAmp tissue kit (Qiagen). Variability was analyzed at six microsatellite loci cloned in *B. glabrata* (Mavarez et al. 2000; Genbank accession number: AF216274, AF216280, AF216275, AF216270, AF216269, and AF216271). Loci AF216274 and AF216280 unfortunately provided ambiguous patterns or were monomorphic and were discarded. Genotyping followed the method described in Mavarez et al. (2000, 2002). Polymerase chain reaction (PCR) amplification of each locus from each species was conducted separately. All PCR products were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Genetic Analyses

Nei's (1987) unbiased mean heterozygosity (H_s) was computed in each sample. The unbiased estimator f of Wright's (1951) F_{IS} was calculated over all loci according to Weir and Cockerham (1984) using FSTAT version 2.9.3 (freely available at: www.unil.ch/izea/softwares/fstat.html). The hypothesis that f significantly differs from 0 was tested using a randomization procedure (Goudet 1995; 15,000 permutations of alleles between individuals in each sample). The estimator θ of F_{ST} (Weir and Cockerham 1984) was calculated over all loci in each sample of both rats and mollusks. Genetic differentiation was tested using the G-based test of Goudet et al. (1996) after 15,000 permutations of individuals between samples; 95% confidence intervals of F_{ST} values were obtained by bootstrapping over loci with FSTAT version 2.9.3. Isolation by distance was tested using Rousset's (1997) method under which a correlation is expected between the logarithm (in a two-dimensional habitat) of the geographic distance and $\theta/(1-\theta)$. The geographic distance between two sampling points was computed as a linear distance. For mollusks, we rejected the measure between DFO1 and DFO2 because samples at low geographic distances are not expected to follow a simple pattern (Rousset 1997). The test was implemented using a Mantel-like test using FSTAT version 2.9.3.

The population structure of schistosomes was investigated once repeated multilocus genotypes have been reduced to single copies within each infrapopulation. Multiple infections of rats by the same schistosome genotype are indeed possible (Sire et al. 2001a; Prugnolle et al. 2002) which affects estimates of genetic distance (Mulvey et al. 1991; Théron et al. 2004). F_{ST} between transmission sites was computed using two methods. First, they were estimated using a hierarchical method. Genetic differentiation was indeed detected among infrapopulations within sites (see Table 1) which should artificially inflated F_{ST} between subpopulations (a schistosome subpopulation refers to the group of infrapopulations within a site). This was done using the software HIERFSTAT developed by J. Goudet (freely available at: http://www2.unil.ch/popgen/softwares/) which implements Yang's (1998) algorithm and computes moment estimators of hierarchical F-statistics (here infrapopulations and subpopulations as hierarchical levels). F_{ST} between transmission sites was tested using a G-based test and randomly permuting 5000 times infrapopulations between transmission sites. Ninety-five percent confidence intervals of F_{ST} values were obtained by bootstrapping over loci. Second, F_{ST} was computed between transmission sites after pooling schistosome infrapopulations. As this value only slightly differed from the one obtained from the hierarchical analysis, it was retained in further analyses.

Comparison of Patterns of Dispersal between Hosts and Parasites

Because parasite dispersal is expected to be mainly dependent on hosts (Jarne and Théron 2001), two populations of hosts that are more connected by migration will also harbor parasite populations that are also more connected. This should translate into two populations of hosts that are less genetically differentiated to harbor populations of parasites that are also less differentiated. In this respect, genetic distances computed between pairs of populations of hosts should be correlated to those computed between parasite populations.

The population genetic structure of the parasite and its two hosts was compared using Cavalli Sforza and Edwards's (1967) chord distance and Nei's (1972) genetic distance. They were preferred to pairwise F_{ST}, because they might better reflect dispersal rate between two subpopulations (Kalinowski 2002), are less sensitive to effective population size (Kalinowski 2002) and because pairwise F_{ST} display high variance (Balloux and Goudet 2002). Nevertheless, pairwise F_{ST} were also used for comparison. These distances were computed between pairs of subpopulations of *R. rattus*, *S. mansoni*, and *B. glabrata* using the MSA software (Dieringer and Schlotterer 2003). Mantel-like tests allowed evaluating the correlation between the chord distances between pairs of populations when comparing pairs of species (implemented using FSTAT vs. 2.9.3). We also tested the correlation between the different distances and pairwise F_{ST} computed between parasite infrapopulations and the shared allelic distance (a measure of genetic distance between individuals) (Bowcock et al. 1994) computed between rats from which these infrapopulations were extracted. The shared allelic distance was computed using MSA. This latter relationship is expected to provide much more power than the previous ones to detect a relationship between the patterns of genetic structure in rats and schistosomes as it uses much more information (distances between each pair of rats and each pair of schistosome infrapopulations).

RESULTS

Genetic Variability and Population Structure

In *B. glabrata*, the number of alleles per locus varied between two and 23 (see supplementary Table S1 for allelic frequencies and allele size available online only at http://dx.doi.org/10.1554/04-522.1.s1). The average H_s over all subpopulations was 0.63 (Table 1). Over all loci and populations, *B. glabrata* populations did not display departures from Hardy-Weinberg expectations ($f = 0.016$, $P = 0.18$; Table 1). The genetic differentiation between sites was high and highly significant (θ [95% confidence interval] = 0.084 [0.052; 0.140], $P < 10^{-4}$). The two populations from Dans Fond displayed a much lower differentiation ($\theta = 0.033$, $P = 0.02$). We observed a significant correlation between $\theta/(1-\theta)$ and the logarithm of geographic distances (correlation coefficient $R = 0.63$, $P = 0.01$; Fig. 2 and Table 2).

In *R. rattus*, the number of alleles per locus varied between five and 12 (see supplementary Table S1 for allelic frequencies and allele size). The unbiased mean heterozygosity (Hs) was equal to 0.60 (Table 1). F_{IS} over all loci and populations was not different from what is expected under Hardy-Weinberg conditions ($f = -0.027$, $P = 0.10$; Table 1). The overall differentiation (θ [95% confidence interval]) among subpopulations was 0.058 [0.039; 0.079] and significantly differed from 0 ($P < 10^{-4}$). No pattern of isolation by distance was detected in rats ($R = -0.12$, $P = 0.72$; Fig. 2 and Table 2).

In *S. mansoni*, the number of alleles per locus varied between two and 19 (see supplementary Table S1 online only, for allelic frequencies and allele size). Within transmission

sites, infrapopulation F_{IS} and F_{ST} computed respectively within and between infrapopulations are presented in Table 1. The unbiased mean heterozygosity (Hs) over all sites was equal to 0.50. The three-level hierarchical F_{ST} between transmission sites computed with HIERFSTAT was high and highly significant (θ [95% confidence interval] = 0.137 [0.090; 0.18], $P < 10^{-4}$). When pooling infrapopulations (one sample per transmission site), the overall differentiation was only slightly increased (θ [95% confidence interval] = 0.145 [0.105; 0.20], $P < 10^{-4}$). No pattern of genetic isolation by geographic distance occurred in *S. mansoni* ($R = -0.19$, $P = 0.60$; Fig. 2 and Table 2).

The genetic differentiation among *S. mansoni* subpopulations was significantly higher than that among rat and mollusk populations (Wilcoxon signed ranked test: schistosomes vs. rats: $P = 0.0039$; schistosomes versus mollusks: $P = 0.002$). A significant difference was also observed between *R. rattus* and *B. glabrata* (Wilcoxon signed ranked test: $P = 0.027$).

Correlations between S. mansoni, R. rattus, and B. glabrata Genetic Distances

Whatever the genetic distance used (Cavalli Sforza and Edwards 1967, Nei 1972, or F_{ST}), no correlation was found between *S. mansoni* pairwise genetic distances computed between subpopulations and those of *R. rattus* and *B. glabrata* (Mantel test: *S. mansoni*/*R. rattus*: Cavalli Sforza and Edwards, $R = 0.60$, $P = 0.06$; Nei: $R = -0.29$, $P = 0.44$; F_{ST}: $R = -0.0139$, $P = 0.96$; *S. mansoni*/*B. glabrata*: Cavalli Sforza and Edwards: $R = 0.44$, $P = 0.23$; Nei: $R = 0.60$, $P = 0.075$; F_{ST}: $R = 0.55$, $P = 0.10$). Nevertheless, we observed a positive correlation between Cavalli Sforza and Edwards's genetic distance computed between parasite infrapopulations and the shared allelic distance computed between rats carrying these infrapopulations ($R = 0.29$, $P = 0.0005$; Fig. 3). This correlation did not depend on the genetic distance used, since a significant correlation was also obtained with F_{ST} ($R = 0.22$, $P = 0.003$) and Nei's genetic distance ($R = 0.21$, $P = 0.0048$).

DISCUSSION

Population subdivision and spatial patterns of both selection and gene flow can influence the coevolutionary process (Thompson 1994). Parasite-host coevolution depends on genetic variation and its structure in interacting species. Therefore, an in depth understanding of population genetic structure of both hosts and parasites is necessary when the coevolutionary dynamics of interacting species is under interest. Patterns and rates of dispersal of all interacting species are particularly under concern (see, e.g., Gandon et al. 1996).

Dispersal Abilities of Host Species

Our results indicate that the *B. glabrata* subpopulations studied here cannot be considered as drawn from the same population. Even geographically close populations appear genetically differentiated. This suggests that dispersal is spatially very limited in *B. glabrata* in agreement with our predictions and previous studies in this species (Mulvey and Vrijenhoek 1982; Langand et al. 1999; Mavarez et al. 2002)
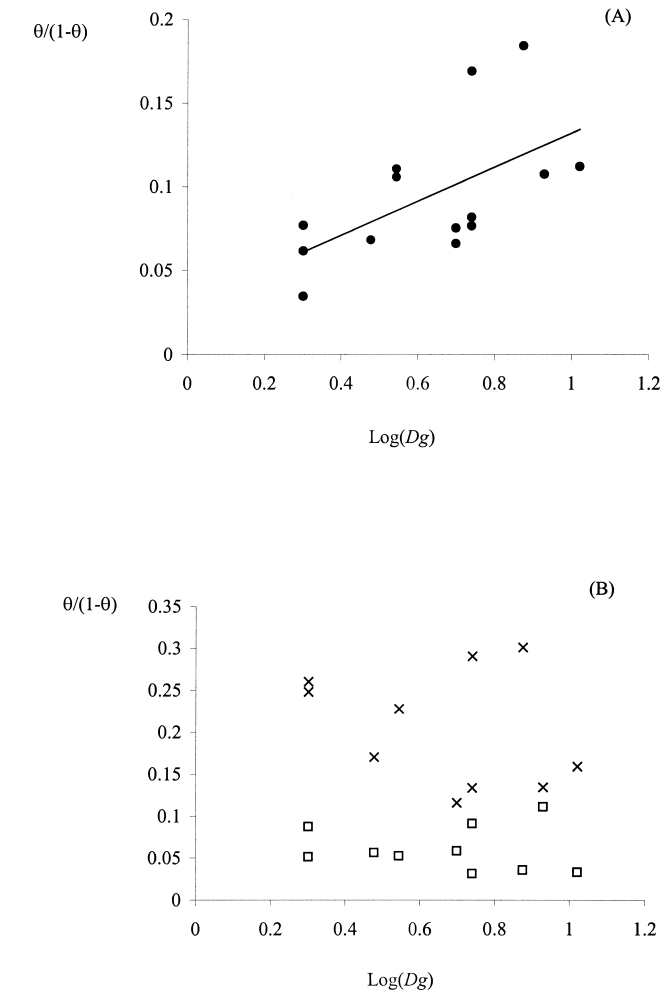

FIG. 2. Relationship between pairwise $\theta/(1-\theta)$ and the logarithm of geographic distance (D_g in km) between transmission sites for: (A) *Biomphalaria glabrata* ($R = 0.63$, $P = 0.01$); (B) *Schistosoma mansoni* (crosses; $R = -0.19$, $P = 0.60$) and *Rattus rattus* (squares; $R = -0.12$, $P = 0.72$).

and in other freshwater snails (Viard et al. 1997; Meunier et al. 2001; Charbonnel et al. 2002). The fact that gene flow is spatially limited is congruent with the observation of a pattern of isolation by distance (see also Mavarez et al. 2002).

The analysis of the population genetic structure of *R. rattus* showed lower genetic differentiation among subpopulations than in snails and no isolation by distance. This suggests more gene flow between subpopulations at regional scale than in snails. These results are somewhat at variance with the demographic results of Delattre and Le Louarn (1981) showing high sedentariness of black rats in the marshy forest of Guadeloupe. Other demographic analyses have shown limited dispersal distances and home ranges in other parts of the world (e.g., Dowding and Murphy 1994; Cox et al. 2000). Discrepancies between demographic and genetic estimates of dispersal are however not extremely surprising, given that long distance dispersal events, which can significantly contribute to gene flow, are difficult to detect using capture-marked-recapture methods (Koenig et al. 1996).

TABLE 2. Pairwise F_{ST} between subpopulations of *Schistosoma mansoni*, *Rattus rattus,* and *Biomphalaria glabrata* in the marshy forest focus of Guadeloupe. Here, mollusk populations DFO1 and DFO2 are pooled.

	JAC	BLP	DFO	GEF	DUB
S. mansoni					
JAC	0				
BLP	0.1753	0			
DFO	0.125	0.1554	0		
GEF	0.2425	0.204	0.1853	0	
DUB	0.1254	0.1138	0.0863	0.1204	0
R. rattus					
JAC	0				
BLP	0.0488	0			
DFO	0.0305	0.0499	0		
GEF	0.0344	0.0835	0.0804	0	
DUB	0.0322	0.0999	0.0553	0.0535	0
B. glabrata					
JAC	0				
BLP	0.0714	0			
DFO	0.0723	0.0943	0		
GEF	0.1556	0.1446	0.0423	0	
DUB	0.1008	0.0971	0.0632	0.0639	0

FIG. 3. Relationship between the shared allelic distance (D_{sa}) computed between individual rats and Cavalli Sforza and Edwards's (1967) genetic distance (D_{cs}) computed between parasite infrapopulations harbored by these rats ($R = 0.29$, $P = 0.0005$).

S. mansoni Dispersal

Few studies have addressed parasite population structure in helminths (see however, Blouin et al. 1995; Nadler et al. 1995; Dybdahl and Lively 1996; Anderson et al. 1998; Sire et al. 2001b; Curtis et al. 2002; Criscione and Blouin 2004; Stohler et al. 2004). However, they suggested that the genetic structure depends not only on parasite-specific characteristics, but also on both host-specific characteristics and on host-parasite relationships, notably with regard to dispersal. Parasites are indeed closely tied to their hosts, and the opportunities for dispersal also depend on the mobility and life histories of hosts (Blouin et al. 1995; Criscione and Blouin 2004). Parasite dispersal should in theory mimic that of the most vagile host (Jarne and Théron 2001).

This led to the prediction that given low prevalence and spatially limited dispersal of mollusks, *S. mansoni* dispersal should largely depend on rats in the marshy focus of Guadeloupe. This prediction is supported by our results. We indeed observed a pattern of isolation by distance in mollusks, but neither in schistosomes nor in rats. Genetic distances between schistosome and mollusk subpopulations were not correlated. No correlation was found between rat and schistosome subpopulations but we observed a significant positive correlation between genetic distances computed between parasite infrapopulations and the shared allelic distances computed between rats carrying these infrapopulations. This is a strong evidence that rats disperse schistosomes between transmission sites as no correlation is expected if rats migrate without carrying any parasite. An alternative although unlikely explanation relies on possible linkage between loci involved in worm-rat interaction and the microsatellite loci used here, assuming genotype tracking between loci controlling infectivity in schistosomes and resistance in rats. However such a linkage seems rather unlikely, given that microsatellite loci were randomly characterized.
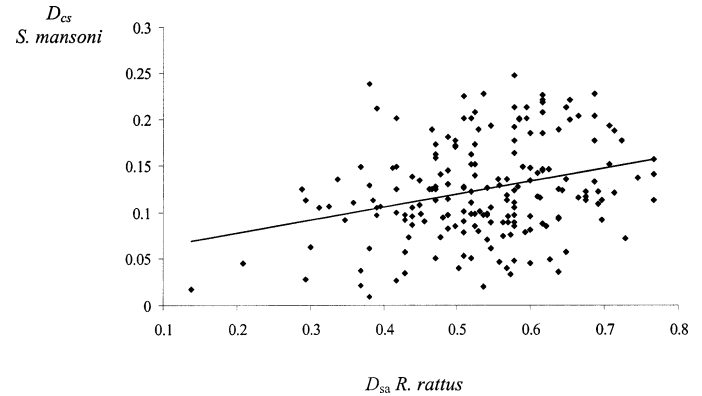
Efficacy of Rat-Mediated Dispersal in Schistosomes

Although schistosomes are clearly dispersed by rats, it remains to be explained why the overall differentiation among parasite populations is twice larger than that among definitive host populations. What could be the biological explanations for such a difference? For the sake of the argument, let us assume that rat and schistosome populations follow an island model of migration and are at the migration/drift equilibrium (e.g., see Hartl and Clark 1997). The larger differentiation in schistosomes than in rats can be translated in $N_r m_r > N_s m_s$ (where N_r and N_s are the effective population size in rats and schistosomes respectively, and m_r and m_s the corresponding migration rates). Given that $N_r < N_s$ (in average, one rat is infected by 24 parasites), we have to explain why $m_r \gg m_s$. $N_r m_r$ can be decomposed in two terms: $N_r m_r = N_r^h m_r^h + N_r^i m_r^i$ (where h and i hold for healthy and infected, respectively). The effective number of migrants in schistosomes is given by: $N_s m_s = I \times N_r^i m_r^i = N_s m_r^i$ where I represents the mean intensity of infection per infected rat. Given that $I \gg 1$, $N_r m_r > N_s m_s$ translates into $N_r^h m_r^h > N_s \, m_r^i = I \times N_r^i m_r^i$. This can be explained by several nonmutually exclusive processes, recalling that parasite populations are probably much larger than host populations.

First, the majority of rats dispersing among transmission sites are not, or weakly, infected (i.e., $N_r^h m_r^h \gg N_r^i m_r^i$). This could occur in several situations. (1) If rats disperse essentially as juveniles, when they are relatively uninfected (Rollinson et al. 1986). (2) If there is a negative relationship between dispersal rate in rats and parasite load. (3) If rats trapped within transmission sites represent a small proportion only of populations and if a large fraction of unobserved rats are uninfected. In such a situation, rats would not disperse parasites very efficiently. No information is currently available in Guadeloupe regarding these three hypotheses, but they could be explored further experimentally. For example, it would be interesting to estimate more precisely the size of local rat populations both within and around transmission sites.

Second, dispersing rats are infected, but parasites display difficulties to establish in the new site ($m_s \neq m_r^i$). This could occur when parasites are locally adapted to their definitive

or intermediate hosts or to other components of the environment. In this case, the effective number of migrants, that is those individuals that fully success to establish and reproduce, will be largely lower than the number of parasites that are carried by hosts.

Dispersal Rates and the Potential for Local Adaptation

Our study showed genetic differentiation in both intermediate and definitive hosts and parasite subpopulations at regional scale. This is an essential feature for the evolution of local adaptation, because resistant and susceptible genotypes in hosts as well as virulent and avirulent genotypes in the parasite are certainly not distributed homogeneously among populations (Gandon et al. 1997). Therefore, this can lead to variable selection pressures between sites and hence to the adaptation of hosts and/or parasites to local environmental conditions.

The ability of parasites and/or hosts to adapt to local conditions depends on their evolutionary potential (Gandon and Michalakis 1996), that is their ability to overcome changes in their environment. The effective migration rate among subpopulations appears to be an essential feature for the evolution of local adaptation (Gandon and Michalakis 2002). Under specific models of infectivity/resistance and for low or intermediate effective migration rates, when parasites migrate more than their hosts within a host-parasite metapopulation, parasites may be more able to infect their sympatric hosts than allopatric ones. By contrast, if hosts migrate more than parasites, these latter are expected to be locally maladapted to their host (Gandon et al. 1996; Lively 1999). We here observed more differentiation in schistosomes than in rats. Under the assumption of migration/drift equilibrium, this should translate into a lower effective migration rate in schistosomes among populations than in rats and hence into maladaptation of parasites to their definitive hosts. The same prediction can be made for the interaction between schistosomes and mollusks given that the genetic differentiation between snail subpopulations is significantly lower than the genetic differentiation between parasite subpopulations.

Obviously, these are only predictions or speculations which have to be substantiated through an experimental approach by testing parasite infectivity over sympatric and allopatric populations of both intermediate and definitive hosts. Indeed, these predictions depend on a particular model of infectivity/susceptibility (the matching allele model) and we currently do not know if the interaction between both host species and schistosomes strictly follows this model. We also have no idea whether these predictions are robust under alternative models such as gene-for-gene or inverse matching allele models. Moreover, the implementation of local adaptation does not depend only on migration but also on many other factors (parasite virulence, mutation rates and generation times of hosts and parasites, and extinction-recolonization processes; Kaltz and Shykoff 1998; Burdon and Thrall 1999; Lively 1999; Thrall et al. 2002) which can preclude to make a good general statement regarding which species should be locally adapted. This is all the more true that these predictions are based on models referring to parasites with direct life cycle. We were here concerned with a parasite species with two obligate hosts, and it remains unclear how local adaptation should evolve in multihost systems. It may presumably be affected by differences in population genetic structure and life-history traits of hosts and in selection pressures exerted by parasites on hosts. The coevolutionary outcome could sensibly differs from that in direct life-cycle parasites, if, for example, there is a trade-off in parasite virulence/infectivity between host species. This certainly requires further theoretical development.

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