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TEST OF PANGAMY BY GENETIC ANALYSIS OF *SCHISTOSOMA MANSONI* PAIRS WITHIN ITS NATURAL MURINE HOST IN GUADELOUPE

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ABSTRACT: Mating system plays a determinant role in the maintenance and distribution of genetic variations. It can be assessed indirectly by analyzing the distribution of the genetic variability within populations or directly by considering how mating pairs are formed. In the present study, 71 pairs of adult *Schistosoma mansoni* worms sampled from naturally infected rats were genotyped to investigate how male and female schistosomes paired according to their genetic relatedness. Among all samples, pangamy, the random association between males and females, could not be rejected. Whereas the schistosome mating system has been intensively studied under experimental conditions, to the best of our knowledge, our study is the first to attempt to understand the way in which males and females pair in natural conditions.

In sexual organisms, genotypes are not transmitted identically from one generation to the next. They are broken up during gamete formation as a result of segregation and recombination processes, being assembled anew in each generation. Thus, the formation of a genotype in newly fertilized eggs results from the union of gametes, which in turn is influenced by mating that takes place between individuals of reproductive age in the previous generation (Hartl and Clark, 1997). Therefore, mating pattern is crucial from an evolutionary point of view because it determines the pattern of gene transmission and genotype formation across generations.

The mating system can be assessed using molecular methods. Usually, it is inferred from indirect approaches by screening the genetic diversity and its distribution in offspring (paternity and pedigree analyses) or in the overall population, e.g., analysis of F_{is}. However, very few studies have analyzed it directly by genetically characterizing individuals of formed pairs (Keller and Waller, 2002), despite the potential effectiveness of such an analysis. This method can notably allow one to directly test pangamy (random association of mates in relation to their genetic relatedness) or alternative hypotheses such as avoidance of kin as mates, a pattern recurrently found in many animals (Pusey and Wolf, 1996).

Schistosoma mansoni is a blood trematode that parasitizes mammals (mainly humans) and is responsible for 1 of the most important human helminthiasis (schistosomiasis) in tropical countries. This is an intriguing parasite with several unusual attributes in this group of organisms (Combes, 1991). For example, it has separate sexes instead of the usual hermaphroditism of most other trematode species. Sexual dimorphism is strongly pronounced in adult schistosomes; the male is larger and holds the female in a groove on its ventral side called the gynaecophoric canal. Mating system in schistosomes is considered as sequential monogamy, as demonstrated through interspecific infections (Tchuem Tchuenté et al., 1996). Once paired, the male and female remain in this state over long periods of time (Combes, 2001). This particularity gives the opportunity to directly collect schistosome pairs from naturally infected definitive hosts and directly analyze the mating system.

In a previous study, Prugnolle et al. (2002) reported a significant genetic differentiation between males and females within each schistosome infrapopulation sampled in the marshy forest focus of Guadeloupe (an infrapopulation corresponds to individuals present within a single individual host). Such a pattern necessarily results in a low genetic relatedness between sexes in each reproductive unit and hence should render the active avoidance of kin as mate unnecessary. Therefore, we can expect that males and females randomly pair within each infrapopulation. In the present study, we specifically collected samples of schistosome pairs and directly tested this prediction in natural conditions.

MATERIALS AND METHODS

Sampling

Samples of Prugnolle et al. (2002) did not permit the analysis of pairs because male and female schistosomes were collected separately. For this reason, we undertook a new sampling effort to collect additional pairs of worms.

In May 2001, 131 rats (*Rattus rattus*) were trapped in the marshy forest of Grande Terre Island (Guadeloupe, French West Indies), where murine schistosomiasis is highly endemic (Théron and Pointier, 1995). To recover adult schistosomes, each rat was perfused following a standard technique (Duvall and Dewitt, 1967); schistosomes were carefully washed in a physiological saline solution and stored in 70% ethanol.

Among these 131 rats, 97 were infected. However, only 3 rats possessed sufficient pairs for genetic analyses. A total number of 71 pairs were collected. The difficulty we had in retrieving pairs was certainly because of the fact that drug doses used to appropriately anesthetize rats also relaxed schistosomes and usually led to the separation of pairs (A. Théron, pers. comm.).

Samples came from 2 different localities: Dans Fond (n = 16 pairs from rat no. 5) and Belle Plaine (n = 20 and 35 pairs from rats nos. 15 and 22, respectively) (see Théron and Pointier, 1995 for a detailed description of the foci).

Genotyping

The protocols followed for DNA extraction and polymerase chain reaction are presented by Durand et al. (2000). Worms were genotyped for 7 microsatellite loci (GenBank AF202965, AF202966, R95529, L46951, and M85305 [Durand et al., 2000]; AF325695 and AF325697 [Curtis et al., 2001]). Acrylamide electrophoretic gels were scored independently by 2 of us (F.P. and P.D.), with samples that raised scoring problems being genotyped again.

Genetic structure analyses

Genetic variability was measured by Nei's (1987) unbiased mean heterozygosity (H_s). Population genetic structure was investigated using Wright's (1965) *F*-statistics. F_{is} measures the within-sample magnitude of departures from Hardy–Weinberg equilibrium expectations, whereas F_{st} measures the genetic differentiation between different samples.

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TABLE I. Genetic characteristics of the 3 *Schistosoma mansoni* infrapopulations. H_s corresponds to the mean unbiased expected genetic heterozygosity (Nei, 1987). Multilocus f (F_{is} estimator) was computed for each infrapopulation. Multilocus $\theta^{M/F}$ (F_{st} estimator) was computed between males (M) and females (F) within each infrapopulation.

	Rat no. 15 (Belle Plaine)	Rat no. 22 (Belle Plaine)	Rat no. 5 (Dans Fond)
Number of pairs analyzed	20	35	16
Number of alleles	2–4	2–6	2–6
H_s	0.36	0.42	0.48
f	−0.18*	−0.08**	−0.045 NS
$\theta^{M/F}$	0.15***	0.06***	0.163***

* $P < 0.01$; ** $P < 0.05$; *** $P < 0.001$; NS: not significant.

These parameters were assessed using unbiased estimators f (for F_{is}) and θ (for F_{st}) of Weir and Cockerham (1984). The significance of departure of F_{is} from 0 was tested by randomizing alleles between individuals in each sample (15,000 permutations), and the probability of obtaining a value as or more extreme than that observed was computed. The significance of genetic differentiation was tested with the allelic *G*-based test (Goudet et al., 1996) using 15,000 permutations of genotypes between samples. Parameter estimates and randomization tests were performed using Fstat V. 2.9.3 (updated from Goudet, 1995).

The mean unbiased genetic variability (H_s) and F_{is} were calculated considering males and females separately in each infrapopulation.

Mating system analysis

Pangamy corresponds to the random pairing of male and female genotypes in relation to their genetic relatedness. To test for random mating, we computed relatedness between individual pairs using the program Kinship V.1.2. (module Relatedness) developed by K. F. Goodnight (http://gsoft.smu.edu/GSoft.html). This software performs pairwise relatedness calculations between individuals in the data set, yielding an unbiased relatedness estimator equivalent to that discussed by Queller and Goodnight (1989).

We then compared the observed relatedness between schistosome couples with the relatedness between all possible male–female pairs within each rat using a Mantel's randomization procedure. The principle of this is as follows: the association between the elements contained in 2 matrices is measured using a statistic (such as the correlation coefficient between corresponding elements), and the significance of this is determined by comparing it with the distribution of the statistic resulting from random reallocation of the order of the elements in 1 of the matrices. In our test, the first matrix corresponded to the genetic relatedness value obtained between all potential male–female pairs of individuals within each infrapopulation. In the second matrix, squares were filled by 0 if it corresponded to a pair observed in our sample, or by 1 for other cases. Under this scheme, we expect a positive correlation coefficient if schistosomes avoid kin as mate more frequently than expected by chance. This test was performed using Fstat V. 2.9.3 by randomizing 15,000 times 1 of the 2 matrices.

To assess the power of the Mantel's test to detect avoidance of kin as mate with our sample sizes, we performed it with artificial samples where avoidance of kin as mate was high. These samples were obtained from observed ones by artificially pairing each male with the less-related female.

TABLE II. Mantel's test correlation coefficient r and percentage of the variance ($100 \times r^2$) explained by the model for the 3 infrapopulations. Results are given for observed and artificial samples. The bilateral P value for each Mantel's test is given.

	Rat no. 15 (Belle Plaine)	Rat no. 22 (Belle Plaine)	Rat no. 5 (Dans Fond)
Observed samples			
r	−0.036	−0.022	0.091
$100 \times r^2$	0.13	0.05	0.82
P value	0.48	0.44	0.15
Artificial samples			
r	0.246	0.302	0.250
$100 \times r^2$	6.1	9.1	6.3
P value	10^{-4}	10^{-4}	2×10^{-4}

RESULTS

Microsatellite polymorphism and genetic structure

Results concerning the microsatellite polymorphism and the genetic structure of the 3 samples are presented in Table I. The maximum number of alleles detected was 6, and the mean unbiased heterozygosity was around 0.42. F_{is} was negative and significant in 2 infrapopulations. Males and females were genetically differentiated in each of the 3 infrapopulations (Table I).

Mating pattern

The mean relatedness (±SE) computed between observed mates was −0.149 ± 0.07 for rat no. 15, −0.03 ± 0.06 for rat no. 22 (both from Belle Plaine), and −0.33 ± 0.1 for rat no. 5 (Dans Fond). In the 3 samples, correlation coefficients (r) between the 2 matrices were low, negative or positive, and not significant (Table II). Therefore, pangamy (the random association of males and females) could not be rejected at the 5% significance level. In comparison, for artificial samples where avoidance of kin as mate was high, the probability to obtain such pattern under the null hypothesis of pangamy was very low in each sample (Table II).

DISCUSSION

Prugnolle et al. (2002) demonstrated a sex-specific genetic structure in *S. mansoni* at a local scale in Guadeloupe: males and females were genetically differentiated within each host. In such a situation, if adults randomly mate, an excess of heterozygosity is expected in offspring compared with Hardy–Weinberg expectations (Prout, 1981). Therefore, this phenomenon opposes the homogenizing genetic effects of inbreeding (the increase of homozygosity in offspring). The evolution of the avoidance of kin as mate is thus unnecessary in such a system.

In the present study, a new sampling effort was made to specifically collect schistosome pairs and directly test this prediction. In each of our samples, males and females were still highly and significantly differentiated, and excess of heterozygotes was observed (mean F_{is} is −0.1). The statistical analysis

of mating patterns by randomization procedures confirmed our predictions. Among all samples, it was never possible to reject the null hypothesis of pangamy. This is unlikely to be due to insufficient statistical power given that correlation coefficients obtained were either positive or negative. Moreover, as demonstrated artificially, the size of our samples seemed sufficient to detect a substantial avoidance of kin as mate. Therefore, we may conclude from our results that in the marshy forest of Guadeloupe, males and females randomly pair according to their genetic relatedness, but that they experience nevertheless an heterogamic reproductive mode because of the genetic differentiation existing between them, resulting in an increased heterozygosity.

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