A study of some characteristics of individual clones of *Schistosoma mansoni* with emphasis on the biological and metabolic activities

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SUMMARY

The variability within schistosome populations was explored using mixed populations of cercariae from multimiracidial snail infections and individual clones of *Schistosoma mansoni* cercariae obtained from monomiracidial snail infections. We investigated the heterogeneity between different clones of *S. mansoni* with respect to infectivity and metabolism. One difference between clones of cercariae was found in the recovery of adult worms from Balb/C mice. Recovery of adult worms was greater after infections with a mixed population than with a clonal population. To investigate some biochemical features of individuals in clones or mixed populations, the uptake of [^{35}S]methionine into individual parasites and their membrane proteins was measured. Isoelectric focusing of a soluble membrane fraction: the frozen–thawed supernatant extracted from individual clones, showed the presence of proteins of isoelectric point between 7·2 and 8·2 in all clones. These proteins were less labelled with [^{35}S]methionine in the clones than in the mixed population. It was concluded that basic proteins are synthesized by all clones and in the mixed population but at different rates. Differences in the rate of incorporation of [^{35}S]methionine into the surface membranes of schistosomula and adult worms derived from individual clones are reported. In addition, a direct correlation between the percentage of recovery of adult worms from mice infected with individual clones of *S. mansoni* and the rate of incorporation of [^{35}S]methionine into schistosomula of these particular clones was observed. It is suggested that the high rate of metabolism shown by an individual clone may account for the enhanced survival of the cercariae derived from that clone during penetration of the skin and migration through the vertebrate host. In order to examine individuals in a population of schistosomula, from a clone or mixed population, the lysosome-specific fluorescent probe LysoTracker DND-99 was used to label the parasites and quantitative fluorescent measurements were made on individual parasites. There were significant differences between clones and a mixed population. Furthermore, the variation between individuals from a mixed population was greater than from that in any clone, just as was found in the infectivity studies. Freshly transformed schistosomula of individual clones labelled with the LysoTracker DND-99 showed less variations in the quantitative uptake of the dye within a single clone when compared to the mixed population. We conclude that for any biochemical and biological parameter, a population of cercariae consists of individuals showing a wide range of values, with a much greater range in a mixed population. This variability is likely to have great relevance for infectivity of the final host and the efficacy of drugs and the immune system.

Key words: *Schistosoma mansoni*, clones, [^{35}S]methionine, LysoTracker, surface membrane.

INTRODUCTION

Individual clones of schistosome cercariae have been studied by several workers (Smith & Clegg, 1979; Al-Adhami, 1981; Wales, 1989; Grevelding, 1999). Variations between populations of the parasite obtained by monomiracidial infections were reported. Variability was also detected even within single clones (Grevelding, 1999). Smith & Clegg (1979) have indicated that the variable levels of immunity to a challenge infection with *S. mansoni* in mice is due to variations in the parasite rather than in the host. This evidence was based upon *in vivo* experiments employing individual clones of *S. mansoni* obtained from infection of individual snails with single miracidia. While the existence of heterogeneity has been established, the nature and the number of factors controlling the expression of such heterogeneity is still unknown (Grevelding, 1999). It was the aim of this study to determine whether such heterogeneity could be demonstrated by individual clones of *S. mansoni* with respect to (i) the infectivity for snails, (ii) the infectivity for mice, (iii) the rate of synthesis of membrane proteins of schistosomula and adult worms and (iv) the rate of the uptake of an

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acidophilic probe (the LysoTracker Red DND-99) specific for labelling of lysosomes.

MATERIALS AND METHODS

Parasites and establishment of the clones

A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and in Balb/C and TO mice. Single miracidia were used to infect individual snails. Snails were shed individually and cercariae were used to infect separate groups of mice percutaneously. Adult worms were recovered by perfusion according to the method described by Smithers & Terry (1965). After perfusion, careful searches were made for any worms remaining in blood vessels of liver and intestine. Experiments were performed on single-sex parasites derived from individual clones of *S. mansoni*. Individual clones were coded according to the batch of snails from which the clone was derived and the snail number in that particular batch. For instance, $C_1(14)$ is the 14th snail from batch number 1. A pool of snails individually infected with 10–15 miracidia was considered as a control group.

Preparation of surface membrane fractions

The freezing and thawing method described by Kusel (1972) was utilized. Frozen worms were allowed to thaw at room temperature. On thawing, the surface membrane partially separated away from the bodies of the worms, but left worm bodies intact. Worms were sieved through muslin cloth and washed twice with PBS, pH 7·4. The membrane filtrate was centrifuged at 40000 ***g*** for 2 h. The soluble membrane fraction termed the frozen-thawed supernatant containing soluble membrane proteins and other proteins was used (Kusel & MacKenzie, 1975).

Radioactive labelling of adult worms and schistosomula with [^{35}S]methionine

The procedure was adapted from the method described by Ruppel & Cioli (1977). Adult worms and schistosomula were labelled by L-[^{35}S]-methionine. Adult worms or schistosomula were pre-incubated in sterile flasks containing 5 ml of Eagle's medium + 10 % FCS at 37 °C for 24 h. After pre-incubation, cultures were examined under the light microscope and the damaged parasites were removed, whereas viable organisms were maintained for labelling. The radioactive labelling was carried out through 2 successive stages. During the first stage, the parasites were incubated in 5 ml of labelling medium deficient in methionine and containing 10 % FCS at 37 °C for 3 h. At the second stage the labelling medium was decanted and a mixture of L-[^{35}S]methionine (250 μCi/ml medium) and 2 ml of labelling medium + 10 % FCS were added to each culture flask. After 18 h incubation at 37 °C, the incorporation of radioactivity was terminated by washing the parasites 5 times in 10 ml of labelling medium containing 30 μg unlabelled methionine/ml of medium. The incorporated radioactivity was determined by trichloroacetic acid (TCA) precipitation.

Isoelectric focusing of the frozen–thawed fractions obtained from labelled adult worms

Adult worms labelled with [^{35}S]methionine were used to obtain the frozen–thawed supernatant fractions as described before. Fifty worms of each of individual clones and 25 pairs of worms of the mixed population were included in this experiment. Samples of 10–20 μl of the frozen–thawed fractions containing 20000 cpm of TCA-precipitated proteins were applied to isoelectric focusing polyacrylamide gels (pH 3·5–10·0). Clear resolution of protein components of all clones tested as well as that of the mixed population were obtained after fluorography. To measure the relative intensities of the isolated bands, a densitometric quantification analysis was performed on the fluorographs using a Joyce Loebl microdensitometer. For convenience in discussion, the bands are numbered sequentially (A1–A10) from pH 3·5 to pH 10·0. Areas of the IEF spectra were determined by using a Delta-T flat bed scanner (Delta-T Devices Ltd, 128 Low Rd, Burwell, Cambridge, England). The scanned images were saved as tagged image files (TIFF) and Delta-T scan image software was used to calculate the spectra areas (Akhkha, 1999).

The incorporation of L-[^{35}S]methionine into adult worms and schistosomula of individual clones

Schistosomula. Schistosomula were prepared by the mechanical transformation method. Three schistosomula preparations of individual clones $C_1(14)$, $C_5(17)$ and $C_6(12)$ and 1 of a mixed population were used in this experiment. Triplicate samples of 0·3 ml aliquots of the schistosomula suspensions (containing 800–1000 schistosomula in Eagle's medium) from each preparation were placed in sterile culture flasks. One ml of Eagle's medium containing 0·5 % lactalbumin hydrolysate + 10 % FCS was added to each flask. After 24 h incubation at 37 °C, 1 ml of fresh labelling medium containing [^{35}S]methionine (specific activity 870 Ci/mmol) at 66 μCi/ml medium was added to each culture flask. Then 0·2 ml aliquots of the incubation medium (containing 123–133 schistosomula) were taken at 2 h intervals.

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The schistosomula were thoroughly washed in labelling medium, then re-suspended in 0·2 ml of PBS and stored at −10 °C. Surfaces of schistosomula were removed by the freezing and thawing method and the frozen–thawed fractions were prepared (Kusel, 1972; Kusel & MacKenzie, 1975). Triplicate aliquots of 50 μl of the frozen-thawed supernatant fractions were tested for TCA-precipitated radioactivity. The counts were expressed as counts/min/1000 schistosomula.

Adult worms. Eight weeks after infection, adult worms were recovered from groups of mice infected separately with 2 male clones: C_1(14) and C_5(17) and a female clone C_6(12). A group of worms obtained from bisexual infection with a mixed population of *S. mansoni* was used as a control group. In this group, worm pairs were used as mixed populations of males and females. After 24 h incubation at 37 °C, 60 viable worms were collected from each group, then worms were distributed into sterile culture flasks. Each culture flask contained 20 worms in 1 ml of labelling medium +10 % FCS. All experimental and control groups were assayed in triplicate. [^{35}S]methionine was added at 60 μCi/ml medium to each culture flask. Four adult worms were removed from each incubation flask at 2 h intervals. Labelled worms were treated as described before with the schistosomula.

Labelling of the schistosomula with the fluorescent probe, LysoTracker Red DND-99

The molecular probe LysoTracker Red DND-99 (Mol. Probes Inc., Oregon, USA) was used (Haugland, 1996). Ten μl of the LysoTracker Red (1 mM solution in dimethyl sulphoxide) were added to 1·0 ml of Glasgow modification of Eagle's medium (GMEM, Gibco, Paisley, Scotland) and considered as the stock solution. Schistosomula were prepared by mechanical transformation of cercariae using the syringe method of Colley & Wikel (1974). One h after transformation, 50 μl of the stock solution of the molecular probe LysoTracker Red DND-99 were added to 1·0 ml of GMEM containing approximately 100 schistosomula. Parasites were incubated at 37 °C for 30 min. After incubation, schistosomula were washed 3 times with GMEM and analysed by quantitative analysis. The amount of fluorescence taken up by the parasites was quantified using a Leitz Orthoplan fluorescent microscope under a 40× objective lens (Modha *et al.* 1997).

Statistical analysis

Statistical analyses were performed using MINITAB 10 programme for Windows version 10·1. ANOVA test was employed to detect significance with confidence interval of 95 %.

RESULTS

Susceptibility of Biomphalaria glabrata *to infections with a single miracidium of* S. mansoni

The susceptibility of *B. glabrata* to infections with a single miracidium of *S. mansoni* was assessed. Batches of 20–30 snails were exposed individually to a single miracidium of *S. mansoni* (Table 1). A total infection rate of 40·9 % (90/220) occurred among the snails that received a single miracidium. The rate of infection in different batches of snails varied from 25 % to 57 %. The figures in Table 1 indicate that the proportion of snails that shed cercariae increased gradually from 7·7 % during the first 8 weeks to 18·6 %, in the 12th week after infection. Results were similar in different batches of snails. Mortality varied between 16 % and 38 %, most of the deaths occurring during the first 8 weeks after infections. In all experiments, the number of emerging cercariae was approximately 50 cercariae per snail in the first 8 weeks. Thereafter, it gradually increased but fluctuated widely through the remaining period. A group of 10 snails, which shed the highest numbers of cercariae (average 500–2000), was selected to obtain clones of cercariae used in the subsequent experiments.

Susceptibility of mice to infections with different clones

In order to determine the sex of worms derived from a single clone of cercariae, separate groups of Balb/C mice were infected with different clones of schistosomes; 80–100 cercariae/mouse were used. Eight weeks after exposure to cercariae the infected mice were perfused and worms were collected. All the worms derived from a single miracidium were of the same sex. Of 10 selected snails, each exposed to a single miracidium, 8 (80 %) were found to have male infections and the remaining snails (20 %) had female infections. The infectivity of individual clones was assessed in a separate experiment, which was repeated 3 times. Four groups of 5–10 mice were given a primary infection of 80–100 cercariae/mouse using the individual clones. An additional group of mice was infected with cercariae obtained from a pool of snails exposed to several miracidia. Adult worms were recovered from the portal system 8 weeks after infection. The ratio of the number of worms recovered from the animals in a group to the total number of cercariae to which the group had been exposed was expressed as the mean percentage recovery of adult worms. Results are summarized in Table 2. There was a small variation between

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Table 1. The percentage of infection and death in snails after exposure to a single miracidium of *Schistosoma mansoni*

		Weeks after exposure to a single miracidium						% Total Snail		
		8		10		12				
Batch	No. exposed	No. shed cercariae	No. died	No. shed cercariae	No. died	No. shed cercariae	No. died	Shed cercariae	Died	Survived no. shedding
I	21	2	2	0	0	10	6	57·1	38·0	4·9
II	24	3	2	7	0	3	2	54·1	16·6	16·8
III	24	3	9	1	1	8	3	50·0	33·3	16·7
IV	39	3	10	1	0	6	2	25·6	30·7	43·7
V	38	1	6	5	1	1	0	26·3	18·4	55·3
VI	38	2	9	3	3	6	2	28·9	36·8	34·3
VII	36	3	7	7	4	7	1	47·2	33·3	19·5
Total	220	17	45	24	9	41	16	–	–	–
%	–	7·7	20·6	10·9	4·1	18·6	7·3	41·3	29·6	27·3

Table 2. The yield of adult worms recovered from Balb/C mice infected with single clones and with a mixed population of *Schistosoma mansoni*

(The results are expressed as the average number of worms recovered (±S.D.) per 100 cercariae inoculated.)

Experiment no.	*Mean percentage of recovered adult worms (±S.D.)				
	C_1(14)	C_5(17)	‡C_6(12)	C_2(4)	Mixed population
I	13·3±8·0 †(10)	1·6±2·1 (8)	6·6±3·8 (5)	10·7±5·5 (7)	25·2±9·7 (5)
II	10·2±5·2 (7)	3·1±3·2 (5)	8·2±6·7 (5)	10·9±3·1 (5)	35·7±10·4 (5)
III	12·1±9·8 (5)	3·7±5·1 (6)	7·4±3·2 (5)	8·3±4·7 (6)	45·0±16·3 (5)
Average	11·9±1·6	2·8±1·1	7·4±0·8	9·9±1·4	35·3±9·9

* Adult worms recovered from groups of 5–10 mice 8 weeks after exposure of each mouse to 80–120 cercariae. The average number of worms recovered from mice infected with the clones varied between 0 and 20 worms/mouse.
†Numbers in parentheses indicate number of mice assayed/group.
‡Female clone.

experiments. This is despite the fact that these experiments were performed at 3 different times with cercariae drawn from different sheds but of the same individual snail. On the other hand, the mean percentage recovery of adult worms from mice infected with cercariae obtained from pools of snails (mixed population) varied considerably (25·2–45 %) in different experiments.

Radioactive labelling of adult worms and schistosomula obtained from individual clones

Adult worms were labelled with L-[^{35}S]methionine. Three groups of worms obtained from mice infected with individual clones and a group of worms from mice infected with a mixed population of *S. mansoni* were used. Fifty worms of individual clones or 25 pairs of worms of the mixed population were selected from each group and labelled with [^{35}S]methionine as described previously. The frozen–thawed fractions were prepared from each group of labelled worms. Results are shown in Table 3. Under the applied culture conditions, the amount of radioactivity incorporated into the frozen–thawed fraction extracted from the pool of 50 worms of individual clones varied considerably. A relatively high rate of incorporation of radioactivity was shown by C_1(14) and C_2(4) compared with C_5(17) and C_6(12). The rate of incorporation of [^{35}S]methionine into the female clone C_6(12) was about 10 times less than that observed with the male clones C_1(14), C_2(4) and C_5(17). The mixed population worms (males and

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Table 3. Trichloroacetic acid precipitation of the frozen–thawed supernatant fractions extracted from individual clones and from a mixed population of *Schistosoma mansoni*

(50 worms were used in the case of individual clones and 25 pairs of worms of the mixed population.)

	TCA-precipitated counts/minute/worm				
Experiment no.	C_1(14) (*males*)	C_5(17) (*males*)	C_6(12) (*females*)	C_2(4) (*males*)	Mixed population (*males*+*females*)
I	41020	20440	5848	54804	79160
2	33412	25364	5016	54104	71382

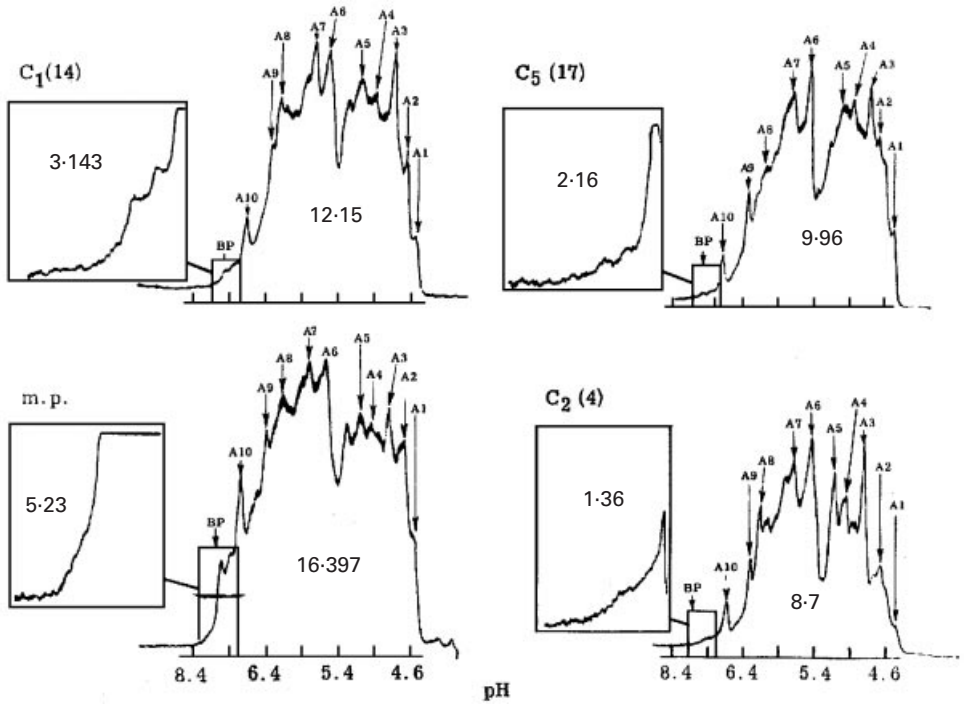

Fig. 1. Densitometric tracings of radio-isotope labelled frozen–thawed supernatant fractions extracted from 50 worms of individual clones and 25 pairs of worms in a mixed population. A1–A10: major protein peaks in each preparation. *Insets*: 5 times magnification of the basic regions of the gels (pH 7·2–8·2). Numbers inside the insets and scanned images indicate the area in cm^2. The spectrum of the inset value used as a percentage is shown in Table 4.

females together) gave the highest incorporation of the radioactive amino acid into the surface membrane proteins of these worms.

The isoelectric focusing spectra of frozen–thawed fractions isolated from adult worms

The IEF spectra of the frozen–thawed fractions obtained from different clones consisted of a regular pattern of 10 major bands (A1–10) ranging in their isoelectric points from pH 3·9 to 7·0 (Fig. 1). The presence of protein bands termed the basic proteins specific to the frozen–thawed fraction was clear. The basic protein bands were located at pH 7·2–8·2. Quantitative differences in the amount of this protein in the frozen–thawed fractions extracted from different individual clones and the mixed population was observed (Table 4). The percentage of the basic protein in the scanned area was 6·379 in the mixed population compared to 3·126, 4·337 and 5·173 in the clones C_2(4), C_5(17) and C_1(14) respectively. Such a result pointed to the possibility of differences in the rate of metabolism among individual clones and this concept was supported in the subsequent experiments.

The incorporation of L-[^{35}S]*methionine into adult worms and schistosomula of individual clones*

Schistosomula. Three schistosomula preparations of individual clones C_1(14), C_5(17) and C_6(12) and 1 of a mixed population were used in this experiment.

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Table 4. The percentages of the basic protein bands in the frozen–thawed supernatant fractions extracted from individual clones and from a mixed population of *Schistosoma mansoni*

(Each number indicates the percentage of the area of the scanned image of basic protein bands related to that of the frozen–thawed supernatant fraction in each spectrum.)

Percentage of basic protein			
C_1(14)	C_5(17)	C_2(4)	Mixed population
5·173	4·337	3·126	6·379

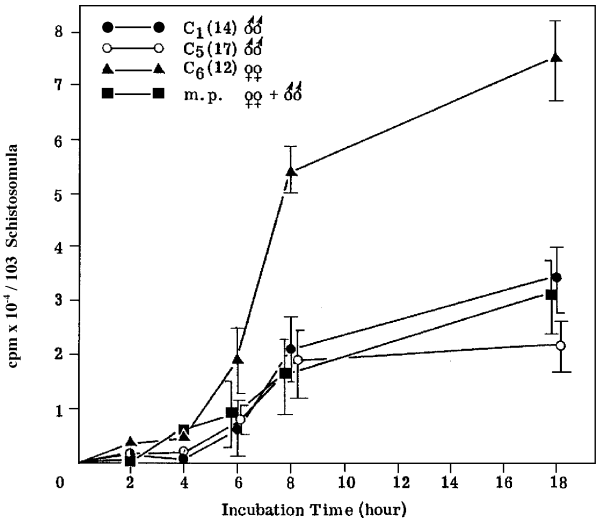

Fig. 2. Incorporation of [^{35}S]methionine into schistosomula of individual clones and mixed population of *Schistosoma mansoni*. Points are mean ± S.D. of 3 different experiments on adult worms derived from clones or mixed populations.

Fig. 2 shows that there is progressive incorporation of the radioactive methionine into the surfaces of schistosomula in all preparations. The rate of incorporation of [^{35}S]methionine significantly increased between 6 h and 8 h of incubation. One striking observation obtained with the female clone C_6(12) was the significant increase in the amount of incorporated radioactivity. A significant difference was consistently found between C_6(12) as compared to C_1(14), C_5(17) and the mixed population. Each of C_1(14) and the mixed population were significantly different from C_5(17) after 18 h incubation time. The results of this experiment showed that schistosomula derived from individual clones incorporate methionine into membrane proteins, but at different rates. This indication in the variability in the rate of synthesis between schistosomula derived from individual clones raised the question whether adult worms of the same clones would demonstrate variations after maturation and sexual differentiation into males and females. In order to answer this question, the rate of incorporation of [^{35}S]-methionine in the groups of adult worms obtained from individual clones was examined as demonstrated by the following experiment.

Adult worms. Eight-week-old adult worms were recovered from groups of mice infected with the same clones used in the schistosomula experiment; C_1(14), C_5(7) and C_6(12). The control group consisted of adult worms recovered from bisexual infection. Fig. 3 shows that the incorporation of radioactivity in all groups of worms was detectable at 2 h after incubation, whereas the incorporation was detected in schistosomula only after 6 h. One interpretation of this observation is that the rate of protein synthesis in adult worms was higher than that in schistosomula. A significant increase in the amount of radioactivity incorporated into the frozen–thawed fraction from the mixed population was evident 6 h after incubation. After that time, a steady increase in the amount of [^{35}S]methionine incorporated was shown by each of C_1(14) and mixed population. [^{35}S]methionine was incorporated at approximately the same rate by C_2(4) and C_5(17). The female clone C_6(12), showed a consistent low rate of incorporation of radioactivity throughout the total labelling period. This result is of interest when it is considered that the data obtained with schistosomula (see last section) derived from the same clone C_6(12) indicated a significant increase in the amount of incorporated radioactivity during the same labelling period and under the same culture conditions (Fig. 2). The data suggest that the rate of metabolism of the parasite relative to others is not necessarily the same during different developmental stages. Nevertheless, results obtained with the other clones C_1(14) and C_5(17) and even with the mixed population showed that both the schistosomula and mature adult stages behaved similarly up to 18 h incubation (compare Fig. 2 and Fig. 3). These results, together with those obtained with the schistosomula, indicated that variations in the rate of metabolism could be demonstrated with different clones of *S. mansoni* either at the schistosomula or at the mature stage.

Labelling of the schistosomula of individual clones with the LysoTracker Red DND-99

To assess the uptake of the LysoTracker by individual clones of the parasite, 4 clones and a control group of mixed population were included in this experiment. Shedding of snails was performed at weekly intervals. Schistosomula obtained by mechanical transformation were labelled with the fluorescent probe. The pattern of labelling showed fluorescence localized in small spots in the parasite. Variability in the fluorescence taken up by schistosomula was quantified by counting 30 schistosomula

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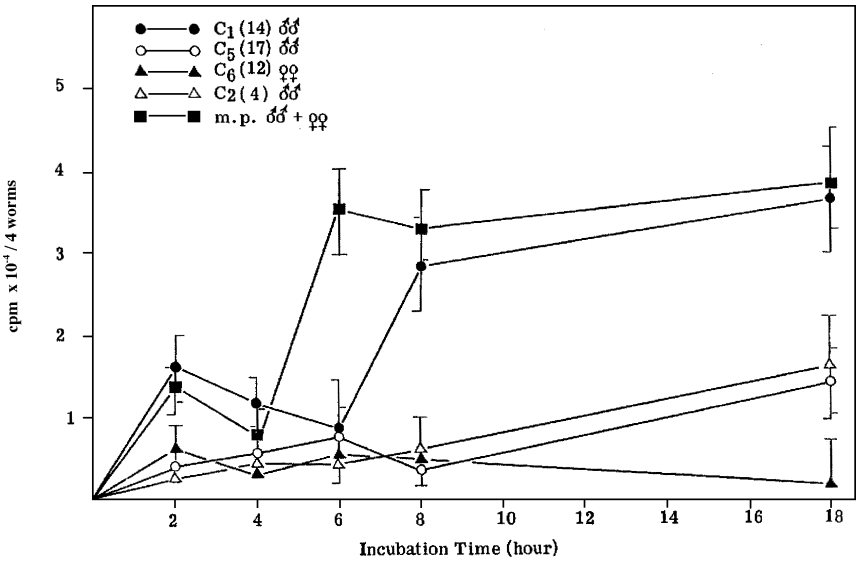

Fig 3. Incorporation of [^{35}S]methionine into adult worms of individual clones and mixed populations of *Schistosoma mansoni*. Points are mean ± S.D. of 3 different experiments on adult worms derived from clones or mixed populations.

Table 5. Uptake of the LysoTracker Red DND-99 by fresh schistosomula obtained from individual clones and from a mixed population of *Schistosoma mansoni*

	Arbitrary fluorescence (mean ± S.D.)			
Shedding no.	C_1(20)	C_2(5)	C_3(4)	Mixed population
1	44·12 ± 4·20	59·30 ± 7·10	24·97 ± 5·76	37·48 ± 11·16
2	33·57 ± 8·28	62·80 ± 2·50	39·76 ± 8·88	44·47 ± 18·36
3	26·06 ± 5·80	25·69 ± 3·19	30·58 ± 4·76	46·75 ± 14·86

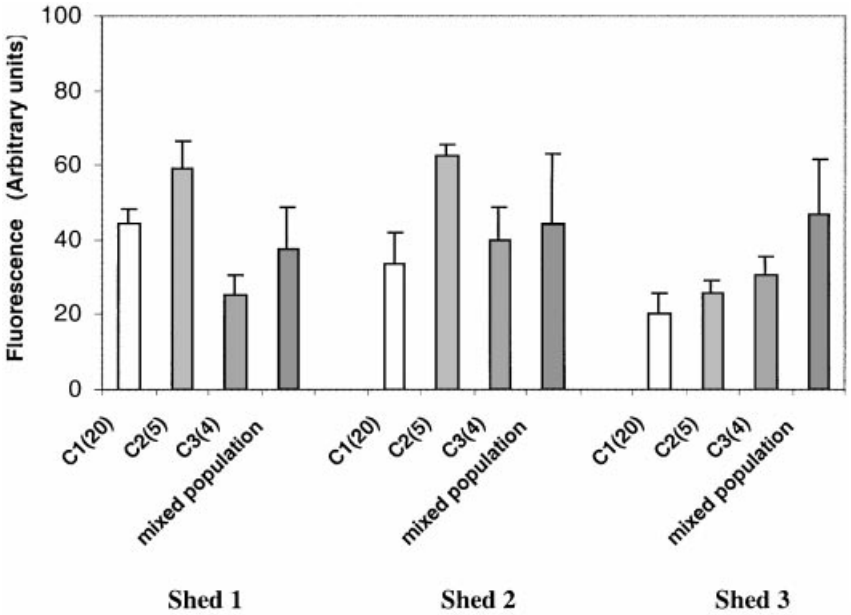

Fig. 4. Uptake of LysoTracker by fresh schistosomula of individual clones and mixed population of *Schistosoma mansoni*.

per each preparation. Results are shown in Table 5 and Fig. 4. The mean amount of fluorescence taken up by individual clones varied significantly in different sheds of the same clone. The mean amount of uptake of the LysoTracker by C_1(20) in the 3 sheds were: (mean ± S.D. 44·12 ± 4·20, 33·57 ± 8·28,

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26·06 ± 5·80) respectively. These results were significantly different ($P < 0·05$). Clone $C_2(5)$ showed no significant differences between the 1st and 2nd sheds (mean ± S.D. 59·30 ± 7·10, 62·80 ± 2·50) respectively, but both were significantly different from the 3rd shed (mean ± S.D. 25·69 ± 3·19). Clone $C_3(4)$ behaved similarly to $C_1(20)$ and showed no significant differences between the 3 sheds of this clone (mean ± S.D. 24·97 ± 5·76, 39·76 ± 8·88, 30·58 ± 4·76) respectively. The mixed population showed no significant differences among the sheds (mean ± S.D. 37·48 ± 11·16, 44·47 ± 18·36, 46·75 ± 14·86) respectively. The interesting observation in this experiment is that the amount of fluorescence taken up by the mixed population exhibits a wide range of variations within the same population in a single shed, as shown by the high standard deviation that ranged between 11·16 and 18·36. Individual clones, however, showed less variation; S.D. ranged between 4·20–8·28, 2·50–7·10 and 4·76–8·80 in $C_1(20)$, $C_2(5)$ and $C_3(4)$ respectively (Table 5, Fig. 4).

DISCUSSION

The hypothesis that antigenic polymorphism among individual clones of *S. mansoni* exists, was first addressed by Smith & Clegg (1979). This hypothesis was based on the evidence that individual clones of *S. mansoni* showed high levels of susceptibility to immunity stimulated by a primary bisexual infection, or were not susceptible at all. Indications of biochemical and genomic diversity among and within schistosome populations were reported (Jones & Kusel, 1989; Grevelding, 1999). No antigenic polymorphism was found and Grevelding (1999) stated that schistosomes may have the ability to generate variability by genome rearrangements which may indicate a molecular basis for the physiological and biochemical variability observed within larval clones. The findings reported in the present work strengthened the above suggestions. The understanding of genetic and biochemical variability among and within schistosome populations is very important as it may explain observed differences in morphology, metabolic properties, disease pathology and immune responsiveness to this parasite.

The establishment of infections in *B. glabrata* appears to be affected by the number of miracidia to which the snails have been exposed. There is evidence indicating a direct relationship between the number of miracidia used for infecting snails and the proportion of snails producing cercariae as a result of infections (Schreiber & Schubert, 1949). Our data indicate that a relatively high rate of infection occurs among snails receiving a single miracidium (40·9 %) compared with the data reported by other investigators; 14 % (Schreiber & Schubert, 1949), 35 % (Stirewalt, 1954) and 12–30 % (Smith & Clegg, 1979). This could be due to the fact that we have tested the snails for up to 12 weeks after exposure and found newly patent snails at the later times. However, this proportion is still much lower than that reported on infecting snails with several miracidia; 70–85 % (Schreiber & Schubert, 1949), 78 % (Stirewalt, 1954) and 70–80 % (Smith & Clegg, 1979). Snails infected with a single miracidium yielded a relatively high number of emerging cercariae. This compares well with the number reported from snails infected with several miracidia (Schreiber & Schubert, 1949). Data are not available for the number of daughter sporocysts produced by a single mother sporocyst inside the snail tissues. Such data would help to explain the high yield of cercariae in both single and multiple miracidial infections.

Variable levels of infectivity as a result of primary infections with individual clones were recorded in Balb/C mice. This finding indicates that variations between cercariae derived from individual clones could result in variations in the level of infections in mice. Kusel & MacKenzie (1975) showed that the frozen–thawed supernatant fraction extracted from adult schistosomes contains surface membrane proteins. In our work, the IEF spectra of labelled adult worms from individual clones were examined. Patterns of the frozen–thawed fractions extracted from individual clones were similar on IEF gels. While the various extracts were qualitatively similar, a quantitative difference was evident. Densitometric tracing and scanning of each extract indicated that the bands representing the basic protein found in the frozen–thawed fraction at pH 7·2–8·2 were more heavily labelled in certain clones than in the others. Furthermore, the frozen–thawed fraction from the mixed population showed more heavily labelled basic protein bands than those obtained from individual clones; the percentages are 6·379 in the mixed population as compared to 3·126, 4·337 and 5·173 in the clones. It is possible that these proteins are synthesized by all clones at different rates, or are synthesized at the same rate, but present in different amounts. Further experiments are needed to clarify this point. Also, the presence of large amounts of this protein in the mixed population compared to the individual clones suggested that it might be synthesized more rapidly as a result of the presence of the 2 sexes together. Molecular studies have shown that expression of some specific genes in schistosome populations requires the presence of male and female worms together (Grevelding, Sommer & Kunz, 1997; Schüßler *et al.* 1998). The IEF pattern from the individual female clone $C_6(12)$ could not be obtained due to the low number of counts demonstrated on labelling adult worms derived from this clone. The other possible explanation for differences in labelling of the basic protein is that the difference could be immune

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response-dependent i.e. due to antibodies having been induced by eggs and cross-reactivity of the antibodies with worm antigens.

Since the rate of incorporation of radioactive amino acids into the surface of schistosomes has been used as a measure of the rate of incorporation of freshly synthesized proteins into the surface (Kusel, 1972), this method was used to compare the rate of synthesis of membrane proteins in adult worms and schistosomula derived from individual clones. We shall consider 2 clones used in all experiments; C_1(14) and C_5(17). Both were male clones. C_1(14) showed the highest percentage of recovery of adult worms and the highest rate of incorporation of [^{35}S]methionine compared with the other clones, whereas C_5(17) showed the least rate of methionine incorporation and a significantly lower worm recovery. This suggests the following hypothesis: the high rate of metabolism shown by an individual clone may account for the enhanced survival of cercariae derived from that clone during penetration of the skin and migration through the host. A result which did not support this hypothesis was that obtained with the female clone C_6(12), as it showed a high percentage of recovery of adult worms, while during the labelling experiment, a very low level of methionine incorporation was observed. The possible explanation for the very low rates of protein synthesis by adult worms of the female clone C_6(12) could be based on the findings reported by other workers. Several studies have indicated that the rate of metabolism among adult females is much higher than that in the males (Zussman, Bauman & Petruska, 1970; Lawrence, 1973). It was suggested that there is a direct relationship between the rate of metabolism and the high rate of egg production in female worms. It is well known that in *S. mansoni*, females do not mature sexually unless in the presence of males (Armstrong, 1965). As adults, female worms of the clone were severely stunted when compared with females in a mixed population. The female worms recovered from 8 weeks infected mice measured approximately 5·25 mm in length (range 3·5–7·0 mm), i.e. slightly less than half of the average length of the corresponding female worms from bisexual infection (6·5–15·0 mm, average 10·75 mm). In an *in vitro* culture system, it has been observed that DNA synthesis declined in separated females and increased when these females were re-mated with males (Den Hollander & Erasmus, 1985). Grevelding *et al*. (1997) provided the first molecular evidence that female schistosomes need a constant stimulus from the male to maintain female-specific gene expression. They supported the classical idea that the expression of female-specific genes is the final result of the male signal. In a further study, Schüßler *et al*. (1998) reported the molecular characterization and localization of a DNA sequence of *S. mansoni* which encodes a putative amidase that is selectively expressed in the gastrodermis of female worms. In eukaryotes, amidase plays an important role in the digestion of protein during the catabolic pathway and may also present an important component of the immune defence strategy, and its localization exclusively in female gastrodermis suggests a role in digestion. However, the precise role of this gene and whether its transcription is regulated by pairing with the male has not been investigated yet (Schüßler *et al*. 1998). Thus, observations reported by other investigators could explain the results obtained with the female clone C_6(12) in our work. The high rate of metabolic activity (as shown by methionine incorporation) of schistosomula derived from this clone may explain the high yields of recovery of adult worms. It is likely that most of the cercariae from C_6(12) would penetrate host skin due to their high rate of metabolism. As these schistosomula establish themselves successfully in the host and mature into adult females, the absence of males may have led to the lack of expression of certain genes that are essential for their metabolic activities as has been demonstrated in the labelling experiments. Although this explanation seems to be acceptable, no firm evidence was found to support this hypothesis. A detailed comparative study at molecular levels between the incorporation of radioactive amino acids in paired and single clones should provide such evidence.

An acidophilic fluorescent probe (LysoTracker Red DND-99 specific for lysosomes in mammalian cells (Haugland, 1996)) was used to investigate the uptake and vesiculation processes involved in fresh schistosomula derived from individual clones and mixed populations of *S. mansoni*. The probe bound to specific acidic vesicles which have all the characteristics of lysosomes and was found to accumulate in the guts of these schistosomula. Results showed that the mixed population exhibited a wide range of variations with respect to the uptake of the fluorescent probe (S.D. 11·6–18·36). Whereas the individual clones showed less variations; the S.D. ranged between 4·28–8·28, 2·50–7·10 and 4·76–8·88 in C_1(20), C_2(5) and C_3(4) respectively. The data indicate that diversity occurs even within clonal cercarial populations but at limited levels when compared to the mixed population. It is concluded that variations within schistosome cercarial populations could be minimized by using individual clones. Grevelding (1999) reported similar observations using a semi-quantitative PCR analysis with DNA of clonal cercariae that originated from monomiracidial snail infections. He stated that the diversity phenomenon may originate from genomic instability being generated in the intermediate host stage. However, further work is being carried out in our laboratory to understand the behaviour of individual clones of *S. mansoni* by using different fluorescent probes.

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The present study has led to the view that individual clones of *S. mansoni* vary with respect to some of their characteristics. Individual clones with a high rate of metabolism could establish better infection in mice than clones with a low rate of metabolism. This finding may indicate an important mechanism utilized by schistosomes in penetration of the host skin. Wilson *et al*. (1978) reported variations in the activity patterns and changes in shape of schistosomula recovered from various locations during migration of *S. mansoni*. Also, it is possible that the variations in the metabolic activities of individual clones could be responsible for protecting the parasite from the immune attack of the host. For instance, the more active clones may acquire host molecules or may shed the surface membrane faster than the less active clones. Further studies into the different biological and molecular aspects of individual clones of *S. mansoni* should yield information that could be of great value in the field of schistosomiasis.

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