PHENOTYPIC DIFFERENCES IN *SCHISTOSOMA MATTHEEI* OVA FROM POPULATIONS SYMPATRIC AND ALLOPATRIC TO *S. HAEMATOBIUM*

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ABSTRACT

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Schistosoma mattheei ova were collected from cattle in different localities in South Africa and after hatching, miracidia were used to infest *Bulinus (Physopsis) globosus*. Cercariae harvested from these snails were used to infest the definitive host *Praomys (Mastomys) coucha* and eggs from the resulting female *S. mattheei* were collected. These ova were compared with a *Schistosoma haematobium* × *S. mattheei* hybrid similarly collected from an infested *P. (M.) coucha*. The results indicate that *S. mattheei* populations which are sympatric to *S. haematobium* possess *S. haematobium* characteristics. It is suggested that the gene pools of populations of the parasite in these areas are infiltrated with *S. haematobium* genes via the *S. mattheei* × *S. haematobium* hybrid originating from human hosts.

INTRODUCTION

Van Wyk (1983) emphasised the importance of the ruminant parasite *Schistosoma mattheei* in the epidemiology of human schistosomiasis. He referred to the work of Pitchford (1959, 1961), who discussed the manner and consequences of hybridization between *S. mattheei* and the human parasite *Schistosoma haematobium*, stressing the necessity of further studies in order to determine the extent of gene exchange between the two species. He also pointed out that, on account of the considerable variation in the prevalence of the infection in man, different geographical strains of *S. mattheei* may have evolved through the incorporation of genes of *S. haematobium* into the gene pool of *S. mattheei* in regions where these two species exist sympatrically.

The present study was undertaken in an attempt to define and describe certain morphological characteristics of *S. mattheei* ova from different localities in the RSA and to assess the value of the overall length and breadth of the egg as well as the breadth at various defined distances from the tip of the spine and posterior end of the egg to distinguish between laboratory-raised representatives of the different isolates.

MATERIALS AND METHODS

S. mattheei was isolated from cattle in or originating from the following localities in the RSA.

1. KaNgwane
2. Northern KwaZulu
3. Humansdorp district (E. Cape Province); this is the type-locality of *S. mattheei* (Veglia & Le Roux, 1929)
4. Ventersdorp district (W. Transvaal)

The KaNgwane and Northern KwaZulu material was obtained from the livers of cattle slaughtered at the Nelspruit and Vryheid abbatoirs respectively. *S. mattheei* ova were extracted from infected livers by macerating a portion of liver in a 0,9 % saline solution and washing the suspension with tapwater through a helminth filter (Visser & Pitchford, 1972).

The *S. mattheei* ova from the Humansdorp and Ventersdorp districts were obtained from infected cattle dung which was also washed through the helminth filter.

The cattle originating from both KaNgwane and KwaZulu were living in areas with high *S. haematobium* endemicities in man whereas both Ventersdorp and Humansdorp are non-endemic areas (Gear, Pitchford & Van Eeden, 1980).

Bulinus (Physopsis) globosus was used as intermediate host and *Praomys (Mastomys) coucha* as the definitive host for all the laboratory colonies of *S. mattheei*.

For comparative purposes, the eggs produced by a F₁ *S. mattheei* × *S. haematobium* hybrid in *P. (M.) coucha* were also studied. The hybrid was obtained from a human patient in the Nelspruit district. This patient voided eggs that were phenotypically those of *S. mattheei* but which reverted to *S. mattheei* × *S. haematobium* in the second generation in *P. (M.) coucha*, a reversion similar to the one described by Pitchford (1961).

The following measurements were made on each ovum (Fig. 1):
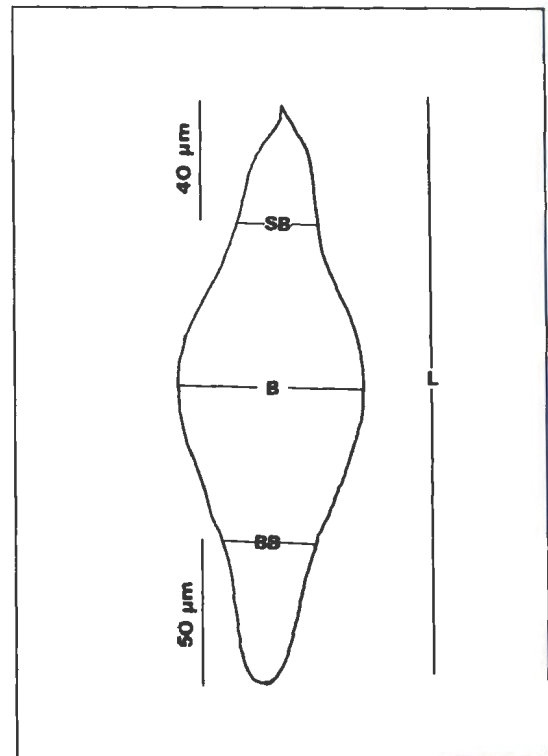
1. Maximum length (L)
2. Maximum breadth (B)

FIG. 1 Measurements made on each ovum [L = maximum length, B = maximum breadth, SB = breadth 40 μm from the tip of the spine and BB = breadth 50 μm from the blunt end (posterior) of the egg]

TABLE 1 Dimensions and ratios of dimensions of the eggs of 4 populations of *S. mattheei* and a *S. mattheei* × *S. haematobium* hybrid

	Population				
	E.Tvl	W.Tvl	E.Cape	N.Natal	Hybrid
No. of eggs recorded	75	75	75	75	75
Length (L) M.* S.D.**	188,5 19,2	218,5 22,5	197,8 16,0	188,0 16,4	169,3 13,1
Breadth (B) M. S.D.	58,8 7,7	64,7 6,9	60,3 5,6	63,5 5,3	64,9 4,9
Breadth 40 μm from tip of spine (SB) M. S.D.	32,9 5,5	27,2 3,0	26,6 5,5	35,8 5,7	50,3 3,8
Breadth 50 μm from blunt end (BB) M. S.D.	38,9 6,4	30,5 5,6	44,3 5,5	45,1 5,3	48,9 4,8
B/L ratio M. S.D.	0,312 0,037	0,297 0,028	0,305 0,029	0,339 0,031	0,384 0,028
SB/L ratio M. S.D.	0,176 0,038	0,125 0,019	0,135 0,031	0,192 0,038	0,299 0,034
BB/L ratio M. S.D.	0,208 0,042	0,142 0,035	0,226 0,038	0,242 0,041	0,291 0,043
SB/B ratio M. S.D.	0,564 0,087	0,423 0,050	0,445 0,102	0,564 0,081	0,777 0,053
BB/B ratio M. S.D.	0,665 0,088	0,475 0,090	0,737 0,078	0,711 0,075	0,754 0,070
SB/BB ratio M. S.D.	0,858 0,143	0,915 0,161	0,610 0,149	0,800 0,128	1,036 0,092

* M. = mean
** S.D. = standard deviation

TABLE 2 Inter-population coefficients of difference (CD) of six ratios in 4 populations of *S. mattheei* and a *S. mattheei* × *S. haematobium* hybrid (for explanation of abbreviations see Table 1)

	W.Tvl	E.Cape	N.Natal	Hybrid
E.Tvl				
B/L	0,230	0,106	0,397	1,107
SB/L	0,879	0,594	0,210	1,708
BB/L	0,857	0,225	0,409	0,976
SB/B	1,029	0,629	0,000	1,521
BB/B	1,067	0,433	0,282	0,563
SB/BB	0,187	0,849	0,214	0,757
W.Tvl				
B/L		0,140	0,711	1,553
SB/L		0,200	1,175	3,283
BB/L		1,150	1,315	1,910
SB/B		0,144	1,076	3,436
BB/B		1,559	1,430	1,743
SB/BB		0,983	0,397	0,478
E.Cape				
B/L			0,566	1,385
SB/L			0,826	2,523
BB/L			0,202	0,802
SB/B			0,650	2,141
BB/B			0,169	1,585
SB/BB			0,685	0,964
N.Natal				
B/L				0,762
SB/L				1,486
BB/L				0,583
SB/B				1,589
BB/B				0,296
SB/BB				1,072

3. Breadth, 40 μm from the tip of the spine (SB); Pitchford (1965) considers this measurement useful for the characterisation of *S. mattheei* ova
4. Breadth, 50 μm from the blunt end (posterior) of the egg (BB); this measurement was taken in order to describe the spindle characteristics of the blunt end of the egg (Alves, 1949).

The mean and standard deviations of each measurement was determined. The following ratios were calculated: B:L, SB:L, BB:L, SB:B, BB:B and SB:BB. With this information at hand the coefficient of difference (CD) of each ratio was computed for all the isolates using the formula:

$$CD = \frac{M_B - M_A}{SD_A + SD_B}$$

where M_B and M_A are the means of the ratio for isolates A and B and SD_A and SD_B the standard deviations of the same ratio.

A mean coeffcent of difference ($\overline{CD}$) was calculated from each set of values.

Commencing with a value of 0 (total similarity between two populations), the greater the value (of CD), the smaller the percentage of overlap between the isolates and the more unrelated they are. The conventional CD value for subspecific differensiation is 1,28 (Mayr, 1969).
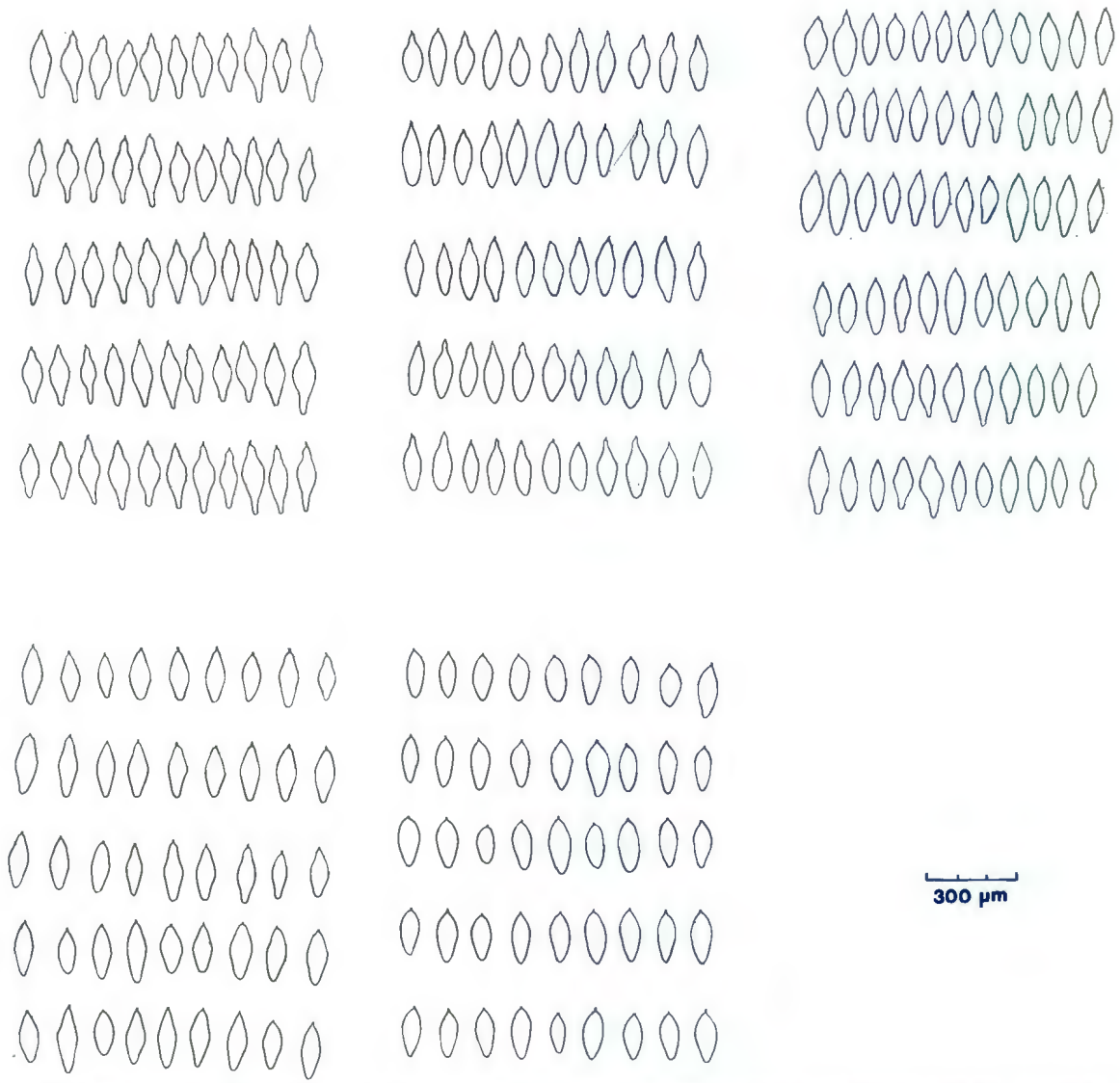
FIG. 2 *S. mattheei* ova from (a) the western Transvaal, (b) the eastern Cape, (c) the eastern Transvaal, (d) northern Natal and (e) from a *S. mattheei* × *S. haematobium* hybrid

TABLE 3 The mean coefficients of difference ($\overline{CD}$) for 4 populations of *S. mattheei* and a *S. mattheei* × *S. haematobium* hybrid

	Hybrid	N.Natal	E.Tvl	E.Cape	W.Tvl
Hybrid	0				
N.Natal	0,964	0			
E.Tvl	1,105	0,252	0		
E.Cape	1,566	0,516	0,472	0	
W.Tvl	2,067	1,017	0,708	0,692	0

RESULTS

Representative ova of the 4 isolates of *S. mattheei* and of the *S. mattheei* × *S. haematobium* hybrid are depicted (Fig. 2a–e), while the means and standard deviations of all measurements and ratios are tabulated (Table 1).

The ova from the western Transvaal, although having the greatest length and breadth, display relatively long and thin terminations (Fig. 2a). Most of the ova from the eastern Cape isolate were characterised by a thick blunt end but with a thin sharp end (Fig. 2b) while those from the eastern Transvaal (Fig. 2c) and northern Natal (Fig.

2d) had ends which taper gradually.

The perimeters of the scatter-grams for the 6 combinations of the 4 measurements are illustrated (Fig. 3a–f). It should be noted that in most of the cases the eastern Transvaal and northern Natal isolates revealed a greater overlap with the hybrid than the other two isolates and of these populations the eastern Transvaal eggs displayed a greater variation in shape.

The mean coefficient of difference (Table 3), as based on the CD values of the 6 ratios (Table 2) are considered to be numerical expressions of the morphological differences.
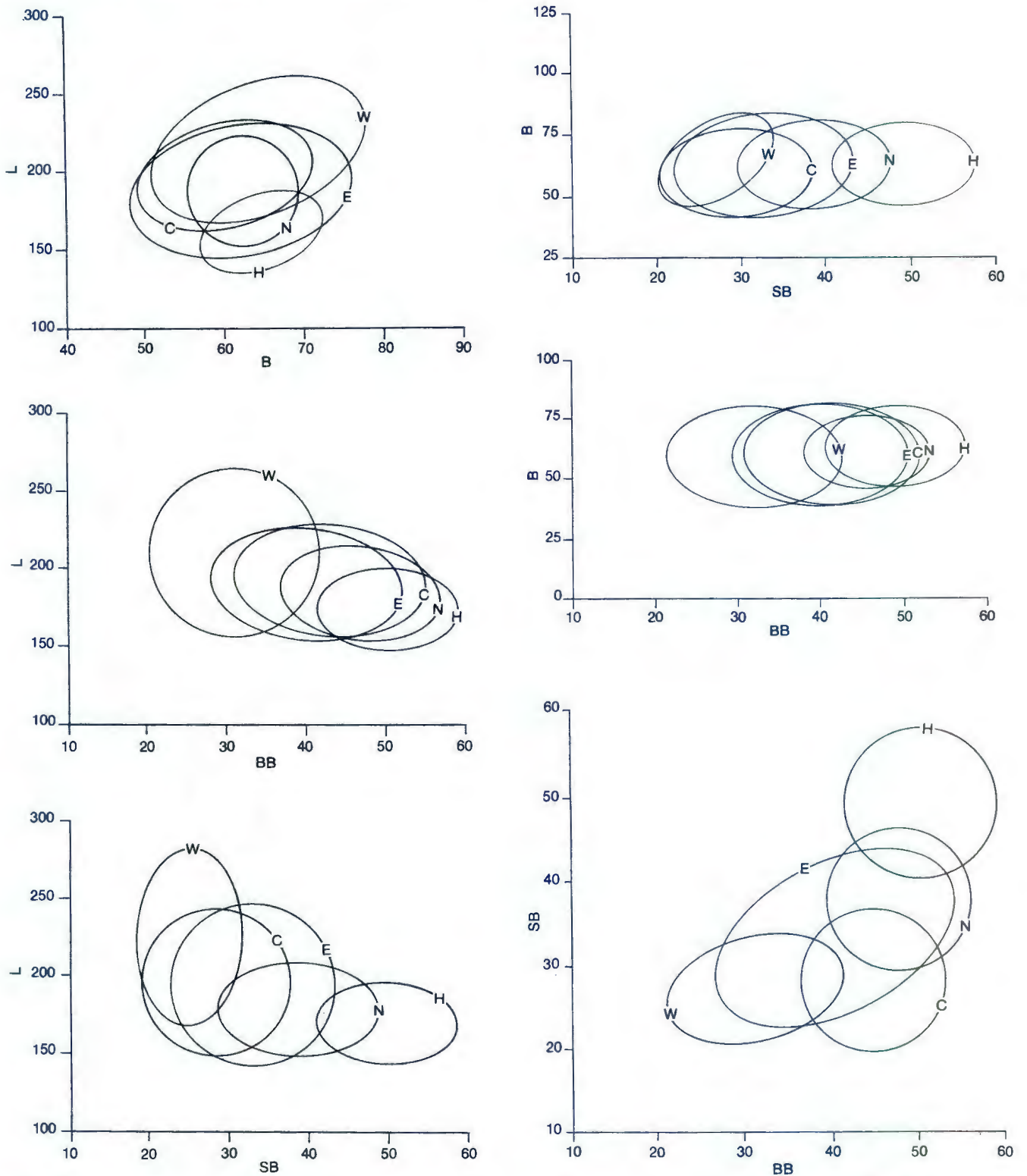
FIG. 3 Perimeters of scattergrams depicting (a) L vs B, (b) B vs SB, (c) L vs BB, (d) B vs BB, (e) L vs SB and (f) SB vs BB (W = western Transvaal, C = eastern Cape, E = eastern Transvaal, N = northern Natal and H = *S. mattheei* × *S. haematobium* hybrid)

DISCUSSION

The statistical analysis ($\overline{CD}$) confirms our subjective interpretation that the four measurements (L, B, SB and BB) of the egg shows the extent to which *S. haematobium* has influenced the size and shape of the eggs of *S. mattheei*. From the illustrations of *S. mattheei* ova (Fig. 2) it is clear that those from northern Natal and eastern Transvaal, where *S. mattheei* and *S. haematobium* exist sympatrically, resemble *S. haematobium* more closely

than the other two populations. This is confirmed by the data presented in Table 3.

The ova from the eastern Cape isolate occupy an intermediate position. This parasite was collected on the perimeter of the distribution range of *S. haematobium*. Humansdorp, the southern limit of the distribution of *S. mattheei*, is situated a quarter of a degree south of Uitenhage, the southern limit of *S. haematobium* (Pitchford & Geldenhuys, 1960). Harley (1864) gave a detailed

description and illustrated the ova found in a urinary infection in a patient living in the Uitenhage district. In retrospect the morphology of the ova clearly shows a mixed infection of *S. haematobium* and *S. mattheei*. According to Harley *S. haematobium* was common in the district in the previous century.

The ova of the allopatric western Transvaal isolate are morphologically distinct in shape and quite remote from those of *S. haematobium* and may therefore be regarded as a "pure" *S. mattheei*.

The results of this study indicate that *S. haematobium* genes are absorbed into the gene pool of certain *S. mattheei* populations in South Africa. Pitchford (1961) demonstrated that a *S. haematobium* × *S. mattheei* hybrid, from a human patient, has the ability to establish a patent infection in a bovine host. This experiment demonstrates the manner in which *S. haematobium* genes are incorporated into the gene pool of a *S. mattheei* population. Where *S. haematobium* and *S. mattheei* exist in the same area the changed morphology of the eggs caused by hybridisation between the 2 species is of such a minor nature—shown by the low interpopulation ($\overline{CD}$) values—that subspecies distinction is not justified between populations sympatric or allopatric to *S. haematobium*.

In order to confirm the present findings, we are currently studying the iso-enzyme and the cercarial shedding patterns, as well as the ultrastructure of the integument of the adults of the four isolates and the hybrid.

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