Phenotypic Plasticity in Adult Worms of *Schistosoma mansoni* (Trematoda:Schistosomatidae) Evidenced by Brightfield and Confocal Laser Scanning Microscopies

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A comparative morphometric study was performed to identify host-induced morphological alterations in Schistosoma mansoni *adult worms. A wild parasite population was obtained from a naturally infected rodent* (Nectomys squamipes) *and then recovered from laboratory infected C3H/He mice. Furthermore, allopatric worm populations maintained for long-term under laboratory conditions in Swiss Webster mice were passed on to* N. squamipes. *Suckers and genital system (testicular lobes, uterine egg, and egg spine) were analyzed by a digital system for image analysis. Confocal laser scanning microscopy (CLSM) showed details of the genital system (testicular lobes, vitelline glands, and ovary) and the tegument just below the ventral sucker. Significant morphological changes ($p < 0.05$) were detected in male worms in all experimental conditions, with no significant variability as assessed by CLSM. Significant changes ($p < 0.05$) were evident in females from the wild population related to their ovaries and vitelline glands, whereas allopatric females presented differences only in this last character. We conclude that* S. mansoni *worms present the phenotypic plasticity induced by modifications in the parasite's microenvironment, mainly during the first passage under laboratory conditions.*

Key words: *Schistosoma mansoni* - phenotypic plasticity - adult worms - *Nectomys squamipes* - C3H/He mice - confocal laser scanning microscopy

Vertebrate animals live in different environments where they are exposed to several parasites. The outcome of this interaction is that both individuals have developed strategies for adaptation, aiming to reach an adjusted relationship (Hart 1992). In order to be established within the host, a successful parasite must possess the ability to tolerate the host immune responses (Zelmer 1998).

The trematode *Schistosoma mansoni* is a parasite worm naturally present in some rodents from Africa (Duplantier & Sène 2000) and Neotropical region (Combes 1990, Théron et al. 1992, Rey 1993, Alarcón de Noya et al. 1997, D'Andrea et al. 2000, 2002). The water-rat *Nectomys squamipes* (Rodentia: Sigmondontinae) acts as a possible reservoir of schistosomiasis mansoni in Brazil (Rey 1993, D'Andrea et al. 2002) and can also be used as an alternative animal model in basic biological studies on schistosomiasis (Rodrigues-Silva et al. 1992, Maldonado Jr. et al. 1994, Ribeiro et al. 1998). Although this parasitism can provoke severe damages to humans, rodents are permissive hosts with life-long infections (Machado-Silva et al. 1997), which do not affect their life span (Rodrigues-Silva et al. 1992) or reproductive capacity (D'Andrea et al. 2000). *N. squamipes* develops peculiar granulomas consisting mainly of large macrophages, many of them full of schistosome pigment, characterizing an exudative-macrophage granuloma type, usually smaller than the equivalent granuloma type in mouse, and they never acquire Symmer's fibrosis pattern (Silva & Andrade 1989, Costa-Silva et al. 2002). Such granulomas have been also observed in the rat *Calomys callosus* (Lenzi et al. 1995).

Parasitic flatworms that have been maintained in different hosts rather than in natural ones have undergone strong morphological changes (phenotypic plasticity) (Mouhaid et al. 1997). *S. mansoni* adult male worms were bigger in *Rattus rattus* than in *R. norvegicus* (Jourdane & Imbert-Establet 1980) as well as in *N. squamipes* than in Swiss Webster (SW) mice. Moreover, they presented a shorter distance between suckers and the number of their testicular lobes was enhanced, when an isolate of *S. mansoni* from *N. squamipes* has been developed in albino mice (Machado-Silva et al. 1994). In the present study we demostrate the occurrence of phenotypic plasticity in *S. mansoni* adult worms induced in two host changes: *N. squamipes* to C3H/He mice and albino mice to N. *squamipes*.

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MATERIALS AND METHODS

Animals - SW and C3H/He mice were supplied by the Animal Facility Center from the Oswaldo Cruz Foundation, Fiocruz, Rio de Janeiro, Brazil, and the laboratory-reared *N. squamipes* (Ns) by the Laboratory of Biology and Control of Schistosomiasis Mansoni, Department of Tropical Medicine, Oswaldo Cruz Institute, Rio de Janeiro (D'Andrea et al. 1996). The animals were kept in polypropylene cages (40 cm x 33 cm) with stainless steel screened covers. All animals received a well-fed diet for mice (Nuvilab CR1, Colombo, Paraná, Brasil) and water ad libitum. Sacrifice of the animals followed standard ethical procedures for laboratory animals (GV-SOLAS 1985).

Experimental design

Wild S. mansoni population - Twelve *N. squamipes* were captured in a transmission area of schistosomiasis mansoni in the municipality of Sumidouro, state of Rio de Janeiro, Brazil (22°02'46"S; 42°41'21"W). They were killed under laboratory conditions and perfused for adult worm recovering (Machado-Silva et al. 1994) (Ibama license 061/2003 – CGFAU/LIC). This parasite population was termed as SN.

Sympatric laboratory-reared *Biomphalaria glabrata* snails, measuring 5 mm in shell diameter, were exposed to ten hatched miracidia collected from the rodent's feces. The snails were kept in glass vials with dechlorinated tap water and fed with fresh lettuce (*Lacttuca sativa*).

Seven-day-old C3H/He mice were exposed individually to cercariae (n 50) shed by the infected snails. After eight weeks post infection, the mice were killed by cervical dislocation and adult worms were recovered by portal-hepatic perfusion, establishing another isolate designated as SR.

Laboratory S. mansoni population - Laboratory-reared *N. squamipes* and SW mice (five to six week old) were infected with four different laboratory worm strains that have been maintained for more than five years under laboratory conditions in *B. glabrata* derived from the respective geographical areas: state of Pará (01°28'03"S; 48°29'18"W), state of Ceará (03°45'47"S; 38°31'23"W), state of Rio Grande do Norte (05°45'54"S; 35°12'04"W), and state of Pernambuco (08°45'48"S; 34°54'47"W). The parasites isolated from *N. squamipes* and SW were designated as BE/Ns, CE/Ns, CM/Ns, and CMO/Ns or BE/Sw, CE/Sw, CM/Sw, and CMO/Sw, respectively. All populations were routinely maintained in SW mice and *B. glabrata* snail host at the Department of Malacology, IOC-Fiocruz, Rio de Janeiro, Brazil.

Nine weeks pos-infection, the animals were killed (cervical dislocation) and perfused for adult worm recovering (Smithers & Terry 1965). This set of experiment was regarded as in allopatric condition, because each worm population came from a different geographical area.

Morphometric analysis of adult worms - The worms were fixed in AFA (alcohol 95%, formalin 3%, and glacial acetic acid 2%), stained with chloride carmine, clarified in methyl salicylate and preserved as whole-mounts (Neves et al. 1998).

Brightfield microscopic (Olympus BX50) images were captured by an analogue camera (Sony, 640 x 480 pixels, RGB) and transferred to a computer containing software for image analysis (Image Pro Plus - Media Cybernetics, US). The following morphological characters were analyzed: oral and ventral sucker areas, distance between them (both sexes); number, area, major and minor diameter, and perimeter of the testicular lobes; area, major and minor diameter, perimeter of the egg and its spine (Neves et al. 1998). All measurements are in micrometers, unless otherwise stated. Five specimens from each population (SN, SR, BE/Ns, and BE/Sw) were also studied by confocal laser scanning microscopy (CLSM) (LSM-410, Zeiss), analyzing the following morphometric characteristics: thickness of the tegument below the ventral sucker (for both sexes); length and width of the first, third, and last normal testicular lobes; length, width, and area of the vitelline glands, and length and width of the ovary. On what refers to the body length, values were those previously reported by Machado-Silva et al. (1995): 9.5 ± 1.3 mm for the wild strain and 6.9 ± 1.55 mm for laboratory strain.

Statistical analysis - The data were submitted to the Student's *t*-test (brightfield microscopy) and Mann-Whitney test (confocal microscopy), and statistical significance was assessed at $p \leq 0.05$. The statistical program SPSS 9.0 for Windows was used to facilitate calculations.

RESULTS

Wild strain - Biometric evaluations of male worms from the SN population showed higher values than those observed in SR, except in relation to the number of testicular lobes. Most differences were considered to be significant ($p < 0.05$), except for the major and minor diameters of the testicular lobes (Table I). Testicular lobes in atypical position (supernumerary) were present in adult worms from both populations. Two specimens (SN population) presented only one supernumerary lobe, while six worms had one, three or six lobes (SR population). The distance between the last testicular lobe and the nearest supernumerary one in the SN population varied from 254.66 µm to 517.24 µm, while in the SR population the occurrence of higher number of supernumerary testicular lobes was followed by longer distance between them. For instance, in case with three and six testicular lobes the distances were 976.63 µm and 1251.80 µm, respectively.

All female characteristics of the SR population were bigger than those from the SN population ($p < 0.05$) (Table I).

Both male and female worms from the SR population examined by CLSM presented all measurements bigger than those from the SN population, except for the thickness of the tegument in females. Only measurements of the ovary and width of the vitelline glands were significantly different ($p < 0.05$) (Table II).

Laboratory strain - Measurements of male allopatric specimens isolated from SW mice were bigger than those from *N. squamipes*, except for the CMO/Sw population ($p < 0.05$). The testicular lobe number was the only character that did not show significant changes between laboratory populations, except for CMO/Sw (Table I).

One (BE/Sw and CE/Sw populations) or two (CM/Sw) supernumerary testicular lobes were found in rats, and three (BE/Sw) or five (CE/Sw) in mice. In the latter

TABLE I

Morphometry (mean and standard deviation) of male and female adult worms of *Schistosoma mansoni* originated from *Nectomys squamipes* and *Mus musculus* analyzed by brightfield microscopy

Characters	Populations													
	SN	SR	p	BE/Ns	BE/Sw	p	CE/Ns	CE/Sw	p	CM/Ns	CM/Sw	p	CMO/Ns	CMO/Sw	p
Males	(n 52)	(n 50)		(n 51)	(n 60)		(n 40)	(n 55)		(n 55)	(n 51)		(n 49)	(n 39)	
Testicular lobes															
Number	7 ± 1	8 ± 1	*a*	8 ± 2	8 ± 2	*b*	6 ± 1	7 ± 1	*b*	8 ± 2	8 ± 2	*b*	9 ± 1	7 ± 1	*a*
Area	39819 ± 8477	34225 ± 8398	*a*	22734 ± 4148	25743 ± 5975	*a*	19540 ± 5068	22801 ± 4910	*a*	23657 ± 6627	24109 ± 5374	*b*	21430 ± 6110	12281 ± 3781	*a*
Major diameter	422 ± 85	391 ± 79	*b*	263 ± 40	301 ± 51	*a*	236 ± 43	260 ± 47	*a*	263 ± 60	291 ± 53	*a*	264 ± 69	190 ± 45	*a*
minor diameter	105 ± 56	91 ± 18	*b*	91 ± 14	103 ± 17	*a*	88 ± 16	91 ± 13	*b*	94 ± 17	86 ± 15	*a*	86 ± 17	71 ± 14	*a*
Perimeter	1073 ± 201	972 ± 176	*a*	684 ± 101	733 ± 127	*a*	613 ± 105	678 ± 115	*a*	691 ± 145	726 ± 121	*b*	686 ± 164	469 ± 93	*a*
Suckers															
Oral area	30040 ± 10187	21959 ± 6903	*a*	24433 ± 7383	19410 ± 5799	*a*	23163 ± 6265	28818 ± 7898	*a*	28508 ± 6762	23717 ± 10173	*a*	28101 ± 8190	10209 ± 5407	*a*
Ventral area	40939 ± 14678	28716 ± 7430	*a*	25721 ± 9748	29639 ± 10845	*a*	23362 ± 6574	32639 ± 6574	*a*	29874 ± 11390	32394 ± 10873	*b*	30127 ± 10039	12850 ± 5010	*a*
Distance	326 ± 93	283 ± 80	*a*	168 ± 73	159 ± 57	*b*	171 ± 81	178 ± 69	*b*	177 ± 61	215 ± 58	*a*	267 ± 113	140 ± 72	*a*
Females	(n 21)	(n 48)		(n 31)	(n 49)		(n 18)	(n 50)		(n 28)	(n 32)		-	-	
Uterine egg															
Area	3093 ± 721	6495 ± 2625	*a*	2663 ± 471	2730 ± 625	*b*	2739 ± 836	2698 ± 825	*b*	3054 ± 426	2882 ± 767	*b*	-	-	-
Larger diameter	103 ± 12	136 ± 28	*a*	97 ± 9	95 ± 8	*b*	103 ± 9	97 ± 12	*b*	104 ± 11	103 ± 11	*b*	-	-	-
Smaller diameter	36 ± 7	55 ± 14	*a*	33 ± 3	34 ± 6	*b*	33 ± 3	34 ± 6	*b*	34 ± 8	34 ± 6	*b*	-	-	-
Perimeter	262 ± 27	378 ± 80	*a*	244 ± 25	245 ± 27	*b*	248 ± 20	241 ± 32	*b*	263 ± 28	257 ± 31	*b*	-	-	-
Egg spine															
Area	109 ± 67	305 ± 197	*a*	134 ± 79	146 ± 78	*b*	128 ± 60	189 ± 100	*a*	100 ± 46	113 ± 68	*b*	-	-	-
Larger diameter	17 ± 4	27 ± 9	*a*	18 ± 6	19 ± 6	*b*	17 ± 3	20 ± 5	*b*	17 ± 4	18 ± 6	*b*	-	-	-
Smaller diameter	7 ± 3	12 ± 5	*a*	9 ± 3	9 ± 3	*b*	9 ± 3	11 ± 3	*a*	7 ± 2	7 ± 2	*b*	-	-	-
Perimeter	47 ± 13	86 ± 31	*a*	51 ± 16	54 ± 18	*b*	50 ± 11	59 ± 16	*a*	46 ± 12	49 ± 18	*b*	-	-	-
Suckers															
Oral area	1401 ± 540	2478 ± 1124	*a*	2024 ± 692	2078 ± 658	*a*	2552 ± 943	2845 ± 644	*b*	1822 ± 605	2372 ± 654	*a*	-	-	-
Ventral area	1441 ± 361	1739 ± 1343	*a*	1606 ± 741	2205 ± 784	*b*	2157 ± 995	2534 ± 753	*b*	1635 ± 551	2398 ± 771	*a*	-	-	-
Distance	138 ± 23	212 ± 55	*a*	132 ± 34	144 ± 31	*b*	150 ± 49	147 ± 44	*b*	160 ± 46	163 ± 44	*b*	-	-	-

Areas: measurements in μm^2; *a*: significant difference; *b*: non significant difference; -: the number of recovered female is not applicable; significant difference ($p \leq 0.05$)

(CM/Sw) and in both types of hosts (CMO/Sw) supernumerary testicular lobes were not detected. In *N. squamipes*, the distance between the last testicular lobe and the nearest supernumerary one varied from 8.4 µm to 237.17 µm, while in mice it changed from 49.24 µm to 1493.19 µm.

Few differences were observed among females from allopatric infra-populations (Table I).

In male and female specimens from BE/Ns and BE/Sw populations analyzed by CLSM, only the area and length of the vitelline glands presented significant differences ($p < 0.05$) (Table II).

DISCUSSION

It is well known that the host exerts a strong influence on the morphological features of adult trematodes (Watson & Pike 1993, Adela-Varelo et al. 1998). The outcome of this interaction is that specimens maintained in other hosts rather than their normal ones display a phenotypic plasticity (Mouhaid et al. 1997). The data herein presented reinforce our previous observations indicating that *S. mansoni* male worms undergone host-induced morphological changes already in their first passage under laboratory conditions (Machado-Silva et al. 1994). Probably, phenotypic plasticity may occur because adult worms are established in an environment other than their normal one.

Despite the fact that phenotypic plasticity occurred either in passages from rat to mouse or vice-versa, it seems that the repercussions of environment modifications are more prominent in wild strain conditions. While wild strain male and female worms were quite affected, lesser significant morphological modifications related to laboratory populations were found. It appears that only one passage in a new host is not enough to induce noticeable phenotype variability in populations that have been cycled for long-term under laboratory conditions. Probably this event is due to a "bottle neck" pressure inducing parasite isolates to clonality. For this reason, BE/Ns, BE/Sw, CE/Ns, CE/Sw, CM/Ns, and CM/Sw populations presented few comparative changes among them in both studied hosts, which were more related to measurements of the larger and smaller testicular diameters. Measurements obtained from female characters, such as uterine eggs, egg spine, and ovary were more or less homogeneous. However, the CMO population did not behave as described above, since all its morphological measurements were deeply altered. Interestingly, the CMO population was isolated from a naturally infected Cricetidae host. It was not possible to determine if the environmental conditions that affected the population variability were favorable or unfavorable to the parasites.

The physiological determinants of the phenotypic plasticity are not known but we suppose that wild rodents can give all physiological conditions (nutritional and hormonal) required to the development and maturation of the parasites. Recent findings have indicated that schistosome development was strikingly impaired in interkeukin (IL-7)-deprived mice (Wolowczuk et al. 1999a). IL-7 seems to favor the parasite responsiveness to host endocrine factors (Wolowczuk et al. 1999b).

This fact raises questions concerning the relationship between phenotypic plasticity and biological or physiological implications. It is more likely that male worms display a more pronounced physiological activity than females do. Some biochemical male-female interplay is required for female's nutrition and sexual maturation (Skelly et al. 1998). Male factors affect very specific cellular events in the female (Kunz 2001). Furthermore, it must be pointed out that male worms are more sensitive to the modifications of the hormonal environment induced by the constitutive expression of IL-7 (Roye et al. 2001).

From the morphological point of view, male worms have developed suckers and a well-developed musculature that allow migration against the portal flow to the mesenteric

TABLE II

Morphometry (mean and standard deviation) of male and female adult worms of *Schistosoma mansoni* originated from *Nectomys squamipes* and *Mus musculus* analyzed by confocal laser microscopy

Characters	Populations					
	SN (n 5)	SR (n 5)	p	BE/Ns (n 5)	BE/Sw (n 5)	p
Males						
Testicular lobes						
Length	71 ± 26	73 ± 9	*b*	77 ± 10	71 ± 11	*b*
Width	46 ± 12	59 ± 11	*b*	43 ± 6	40 ± 3	*b*
Tegument – thickness	8 ± 1	8 ± 2	b	11 ± 2	12 ± 3	b
Females						
Vitelline glands (n 10)						
Area	483 ± 139	569 ± 134	*b*	898 ± 271	501 ± 130	*a*
Length	41 ± 8	43 ± 9	*b*	55 ± 13	40 ± 4	*a*
Width	13 ± 2	16 ± 2	*a*	19 ± 1	17 ± 5	*b*
Ovary						
Length	306 ± 25	370 ± 40	*a*	364 ± 89	405 ± 100	*b*
Width	83 ± 5	113 ± 13	*a*	132 ± 27	115 ± 12	*b*
Tegument – thickness	3.5 ± 0.5	2.9 ± 0.6	b	2.5 ± 0.8	2.4 ± 0.8	b

Areas: measurements in μm^2; *a*: significant difference; *b*: non significant difference; significant difference ($p \leq 0.05$)

and colonic venules. In this niche, female worms begin oviposition (Mair et al. 1998, Morand & Müller-Graf 2000). Consequently, any morphological change could affect egg-laying. Obviously, these questions still remain to be elucidated.

Although wild strain and laboratory strain worms displayed few significant morphological differences related to their reproductive system, as assessed by CLSM, our results demonstrated that all measures became bigger after passages from wild rodent to mouse or from mouse to wild rodent hosts. When body size was compared to those obtained by Machado-Silva et al. (1995), the results showed to be similar.

The unusual finding of supernumerary testicular lobes confirms our previous studies both in mice and *N. squamipes* (Machado-Silva et al. 1994, Neves et al. 1998). However, a detailed study by CLSM failed to demonstrate any evidence that this atypical structure is an active component of the reproductive system, once they are isolated and without expression of spermatogenesis (Machado-Silva et al. 1998). New morphological studies (transmission electron microscopy, immunohistochemistry and other) are still required to clarify their functions.

Both the reproductive system and the development of adult male worm tegument in *S. mansoni* depend on the physiological characteristics of the host, such as nutritional and hormonal states. Thus, maturation of the tegument is delayed or does not occur in a non-permissive host (*R. norvegicus*) (Senft et al. 1978). However, if worms are transplanted to a permissive host their normal somatic development is recovered (Cioli et al. 1977). It was not purpose of this current investigation to study the structural organization of the tegument, since both used hosts are permissive, assuring a full somatic development (Machado-Silva et al. 1994, 1997). Probably, this explains why no significant differences were found in relation to the thickness of the tegument either in male or female worms evaluated by CLSM. Nevertheless, little is known about a possible correlation between the thickness of the tegument and the physiological conditions of the host. Even if the host is a permissive one, his nutritional state can influence the development of the parasite. Host diets deficient in protein can lead to reductions in body size in adult worms (Poulin 1996). Recently, we have demonstrated that male adult worms isolated from mice with protein deficiency display thinner tegument than well-fed controls (Neves et al. 2001).

The results here presented confirm the occurrence of phenotypic plasticity in *S. mansoni* adult worms, as previously described by scanning electron microscopy in isolates of the same species and in *S. margrebowie* and *S. mattheei* (Kruger et al. 1988, Machado-Silva et al. 1994). In organisms inhabiting heterogeneous environments, genotypes can produce different phenotypes under different conditions (Poulin 1996). We would like to highlight that phenotypic plasticity is a phenomenon that should be considered by those researchers related to trematodes' systematic (Monis 1999). Thus, if one recovers a same parasitic flatworm from several hosts with morphological variability among them, it does not mean that the isolates are different species. Such results could be only due to genotypes, producing suited phenotypes under adverse physiological conditions, such as a host different from the usual one. However, it is still unknown how genotypes control such phenotypes. There are strong biological, evolutionary and immunological arguments for predicting extensive polymorphism among helminth parasites, but relatively little data and few instances from which the selective forces acting on parasite diversity can be discerned. The study of helminth polymorphisms across the whole species in the wild, and driven by many possible forces from host, vector and environment, is sure to provide many such fascinating and insightful advances and make an essential contribution to the control of helminth diseases (Maizels & Kurniawan-Atmadja 2002). Last but not least, linking proteome and genome could clarify this issue (Asthon et al. 2001), contributing to a better understanding of the occurrence of possible alterations.

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