Schistosoma mansoni: Onset of chemoattraction in developing worms

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Summary. Heterosexual chemoattraction studies on juvenile worms showed that 20- and 21-day-old worms do not attract each other, whereas 23- and 28-day-old worms are attracted to each other and pair with worms of the opposite sex. This onset of chemoattraction in vitro corresponds to the time when worms begin pairing in vivo. The ability of single worms to locate each other and mate is presumably mediated by chemoreceptors.
Key words. Schistosoma mansoni; chemoattraction; worms.

Several species of the genus *Schistosoma* cause disease in a variety of mammals, including humans. Schistosomes infect when larvae called cercariae penetrate mammalian skin and transform into immature schistosomes, which mature and mate within host blood vessels, where female worms deposit large numbers of eggs which invoke the disease process. Until recently, mechanisms by which schistosomes find their mate (i.e. chemoattraction) within the host have not been studied. Studies on the mechanisms of chemoattraction in schistosomes have broad implications for the control of schistosomiasis, because the development of chemical analogues to chemoattractants, or vaccines made against them would prevent mating and therefore egg production. Cessation of egg-laying would not only eliminate most of the schistosome pathology[1–3], but would also interfere with the completion of the parasite life cycle.

Recently, in vitro studies have demonstrated that schistosome adults attract each other in vitro and the attraction is chemically mediated[4,5]. After mechanical separation, adult worms pair more frequently with their original partners than with worms separated from different pairs[4,6]. Worms exhibit greater attraction to one, than to two or three worms of the opposite sex, or to worm pairs[5,7]. Previous studies have all been done using adult worms which were separated from worm pairs. We now report on studies to determine the time of onset of chemoattraction in developing worms.

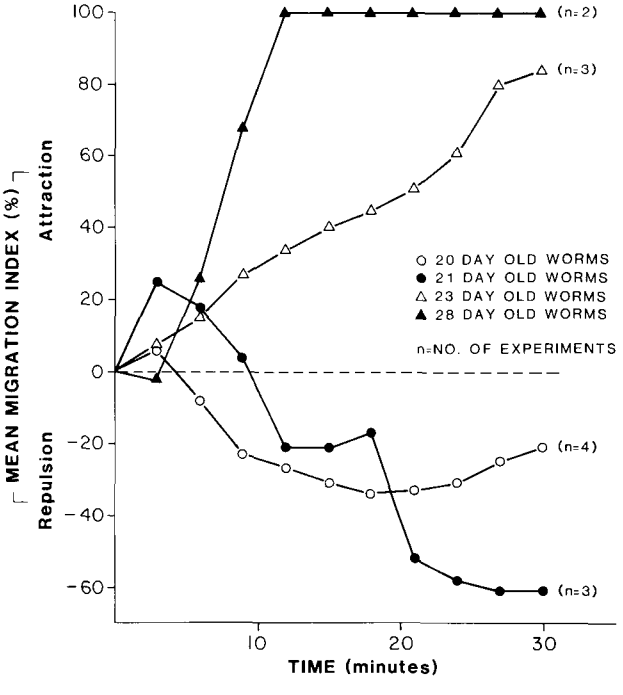
Materials and methods. The parasite. A Puerto Rican strain of *Schistosoma mansoni* (PR-1) maintained in CF-1 mice and an albino M-line of *Biomphalaria glabrata* snails were used. Mice were infected percutaneously by applying approximately 100 cercariae on coverslips to the shaved abdomens, and killed by injecting with 0.3 ml of a 1:1 mixture of 50 mg/ml Nembutal and 1000 U/ml sodium heparin. Worms were recovered by perfusion[8] in 4 °C Earle's balanced salt solution (EBSS) containing 0.1% glucose and 0.5% lactalbumin hydrolysate. Male and female worms were maintained separately in EBSS at 4 °C for approximately 10 min before use in the bioassay.

Chemoattraction bioassay. The bioassay system used was described previously[4,5]. One male and one female were pipetted into each bioassay channel, with initial distances between worms varying from approximately 6 to 15 mm. Worm migration was studied using a closed-circuit video system which consisted of an RCA black and white 1-inch Cohu Low Light camera # 4410 with a 25-mm f1.4 lens, a 20-mm extension tube, and a Panasonic NV8200 video cassette recorder. Distances between worms were measured at 3-min intervals over 30 min. Migration indices were calculated as follows: X-Y/X × 100, where X is the original distance between worms and Y represents distance between worms at a given observation time. The data were analyzed using Kruskal-Wallis one-way ANOVA (TRUE EPISTAT™, Epistat Services, Richardson, Texas).

Results. Distances between worms at each time point are shown in table 1, and mean migration indices are illustrated in the figure. In three of four experiments with 20-day-old

Table 1. Distance (MM) between worms (mean ± SEM)

Time (min)	Age of worms (days) (n = numbers of worms)			
	20 (n = 8)	21 (n = 6)	23 (n = 6)	28 (n = 4)
0	6.85 ± 1.68	7.13 ± 2.75	15.06 ± 1.64	5.50 ± 0.00
3	6.52 ± 2.10	5.66 ± 2.90	13.93 ± 1.59	5.60 ± 0.84
6	7.62 ± 2.39	5.80 ± 2.58	12.63 ± 2.34	4.05 ± 0.35
9	8.95 ± 3.21	6.30 ± 3.13	10.96 ± 3.32	1.75 ± 2.47
12	9.27 ± 3.36	6.90 ± 4.30	10.00 ± 3.15	0.00 ± 0.00
15	9.60 ± 3.53	6.70 ± 4.43	9.16 ± 3.09	0.00 ± 0.00
18	9.82 ± 3.90	6.46 ± 4.56	8.36 ± 3.12	0.00 ± 0.00
21	9.75 ± 3.94	8.33 ± 6.68	7.56 ± 3.43	0.00 ± 0.00
24	9.65 ± 4.01	8.66 ± 7.27	6.16 ± 3.58	0.00 ± 0.00
27	9.27 ± 3.95	8.76 ± 7.51	3.33 ± 3.49	0.00 ± 0.00
30	9.00 ± 3.91	8.83 ± 7.47	2.60 ± 2.71	0.00 ± 0.00



Chemoattraction in 20- to 28-day-old worms. Observations were recorded at 3-min intervals using a closed circuit video recording system and the mean migration index was calculated as described in 'Materials and methods'.

Table 2. Differences between migration patterns of developing worms

Worm groups	p*
20 & 21 days old	0.8205
21 & 23 days old	0.0005
23 & 28 days old	0.0204
20 & 28 days old	0.0001
20 & 23 days old	0.0001
21 & 28 days old	0.0002

* Kruskal-Wallis one-way ANOVA.

worms, the distance between worms equaled or exceeded the initial distance at all time periods. In one experiment worms migrated to one-half their initial distance during the first three minutes, but then moved apart. Attraction was observed in two of three experiments with 21-day-old worms, and one worm pair made contact. In the third experiment, the 21-day-old worms moved away from each other. Attraction was pronounced in all of six experiments with 23- and 28-day-old worms, and five of the six worm pairs made contact.

Differences between the migrations of 20- and 21-day-old worms were not significant (table 2). However, differences between the migrations of 20-day-old worms and 23- or 28-day-old worms were highly significant, as were those between 21 and 23, and 21 and 28 days. Also, the migrations of 23-day-old worms differed significantly from worms 28 days old.

Discussion. These data clearly demonstrate that in vitro attraction begins when *Schistosoma mansoni* males and females are approximately 23 days old, which is in general agreement with reports of pairing in vivo. Standen [9] reported worm-pairing on day 30 when mice were infected with both male and female cercariae simultaneously and day 23 when mice previously infected with female cercariae were superinfected with male cercariae. Wilson [10] reported that pairing in vivo takes place between days 28 and 35 post-infection.

The initial distances between worms varied between experiments because when the small worms were pipetted into the bioassay channel there was an initial drifting related to early fluid disturbance. This also provides another insight into worm behavior because it demonstrates that worm responses can occur over varying starting distances. This variable has not been tested before. It is also of interest that in experiments with 20- and 21-day-old worms the initial starting distances were less than for 23-day-old worms, and attraction only occurred in the latter. To assess worm responses, the formula used [11,12] expressed a mean migration index (MMI), which reflected both positive and negative responses. Inasmuch as a positive MMI reflects attraction, we propose that a negative MMI reflects repulsion. Such a negative interaction has been noted before [7].

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Differences in chromosome A arrangement between *Drosophila madeirensis* and *Drosophila subobscura*

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Summary. The proximal half of the A (= X) chromosome of *D. madeirensis* has a gene arrangement very similar to the A1 or A6 inversions found in *D. subobscura*. Polytene chromosome analysis of hybrids between *D. madeirensis* and strains of *D. subobscura* homozygous for such inversions shows, however, that *D. madeirensis* has a gene arrangement different from any known for *D. subobscura*. These results provide evidence for a greater differentiation of the X chromosome in these species than has previously been described; it seems that the X chromosome is the only one that has undergone structural variation during the speciation process.
Key words. Drosophila-related species; chromosome arrangements; hybrids; speciation.

D. madeirensis [1], an endemic species of the island of Madeira, belongs to the obscura group, being closely related to *D. subobscura*. Krimbas and Loukas [2], studying chromosomal homologies between these species, based on analysis of F1 female hybrid larvae and using as male parents individuals from a strain of *D. subobscura* with the A (= X) standard gene arrangement, observed that the proximal half arrangement of the *D. madeirensis* A (= X) chromosome (segment I) is identical to the A1 gene arrangement of *D. subobscura*. *D. subobscura* has another inversion very similar to A1 in this chromosomal segment, the A6 inversion which is always found in association with A2 inversion located in segment II.

In order to ascertain unambiguously whether the proximal end of chromosome A of *D. madeirensis* has an arrangement which is identical to any one of those found in *D. subobscura*, crosses between *D. madeirensis* and two homozygous strains of *D. subobscura*, A1 and A2 + 6 respectively, have been carried out and polytene chromosomes of female hybrid larvae have been analyzed.

Female hybrid larvae were obtained by crossing *D. madeirensis* females with *D. subobscura* males. Polytene chromosome preparations were obtained from salivary glands of 3rd late instar larvae and stained with lacto-aceto-orcein (acetic orcein 70%, lactic acid 30%). The sections and sub-