# INTERACTION BETWEEN *BULINUS GLOBOSUS* AND *CLEOPATRA FERRUGINEA* AT A TRANSMISSION SITE OF SCHISTOSOMIASIS IN KWALE, KENYA

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**Abstract**: Snail survey data collected in a small village in Kenya from April 1984 to March 1991 were used to analyze the interaction between *Bulinus globosus* (intermediate host of *Schistosoma haematobium*) and *Cleopatra ferruginea* (unsusceptible snail). An inverse correlation was observed between the two snail populations. This finding leads to the suggestion that *C. ferruginea* has limiting effects on *B. globosus* population. The relative penetrative activity of *S. haematobium* miracidia into the two snail species was also examined. Miracidia penetrated *C. ferruginea* as well as *B. globosus*. Although selective mass-chemotherapy has been repeated every 2 years in our study area, the low infection rates in *B. globosus* were recorded in the year when large numbers of *C. ferruginea* and small numbers of *B. globosus* were collected. Therefore, *C. ferruginea* seems to diminish the transmission of *S. haematobium*; *C. ferruginea* reduce the number of *S. haematobium* miracidia which reach to *B. globosus*.

Keywords: Bulinus globosus, Cleopatra ferruginea, Schistosoma haematobium, snail, biological control, antagonist, decoy

### INTRODUCTION

The control of snails that serve as intermediate hosts of schistosomes is an effective means of reducing the transmission of schistosomiasis. However, the use of chemicals to control snails may have undesirable effects on the environment. Therefore, we have become interested in the use of biological control agents that are both environmentally less hazardous and cost effective. Of all biological control strategies, intermolluscan competition is one of the most attractive mechanisms. The ideal biological agent would be able to persist in the habitat of the target snail and induce substantive longterm depression or eradication of the target snail population. Some potential competitor snails which have received considerable attention are *Helisoma duryi*, *Marisa cornuarietus* and *Thiara granifera* (WHO, 1984).

A research program on urinary schstosomiasis has been carried out in a small village in the Kwale District, Kenya since 1981. In our study area, there were some spots where only two snail species, *Bulinus globosus* (intermediate host of *Schistosoma haematobium*) and *Cleopatra ferruginea* were found. The latter species does not serve as an intermediate host for schistosomes or other trematodes of medical or veterinary significance in Kenya (Brown, 1980). We therefore decided to analyze the data obtained so far to examine whether intermolluscan competition occurs at natural snail breeding sites.

As another measure of biological control of schistosomiasis, it has been demonstrated that the infection rate among intermediate host snails exposed to schistosome miracidia was reduced if unsusceptible snail species were present. Such snails apparently prevent miracidia from reaching the intermediate host snails (Chernin, 1968; Upatham and Sturrock, 1973; Laracuente *et al.*, 1979). To determine if *C. ferruginea* has the potential to serve as a miracidial sponge, the present study also examined the relative penetrative activity of *S. haematobium* miracidia into the two snail species.

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## MATERIALS AND METHODS

The study area, Mwachinga village, is located in Kwale District, Coast Province, Kenva. A general description of the study area was given in Noda et al. (1988). The Kadingo River flows through the village. It is less than 5 m in width. Some parts of the river dry up in the dry season, leaving small pools. Towards the end of the dry season, these pools sometimes dry up completely. Snail surveys were carried out twice each month at 7 sites along the river. Villagers had frequent contact with water at these sites. The sites were identified as 18B, 18C, 19, 21, 22, 23 and 24. Snails were sampled for 10 minutes by one person using a doublelayer steel net scoop (4 mm mesh). The number of each species of snail collected was determined and recorded. B. globosus were then put into small petri dishes filled with 2 ml of dechlorinated tap water. The petri dishes were kept in a lighted place for more than 2 hours, and the presence of cercariae of S. haematobium was determined under a stereoscopic microscope. Climatic conditions may be classified into four seasons; a long rainy season from April to June, a cool dry season from July to October, a short rainy season in November, and a hot dry season from December to March. Each study period ran from April to March. Snail survey data collected from April 1984 to March 1991 were used to analyze the interaction between the species.

The relative penetrative activity of S. haematobium

miracidia into *B. globosus* and *C. ferruginea* was examined in the laboratory. *S. haematobium* eggs were collected from school children at Tserezani Primary School in Kwale District. *B. globosus*  $(13 \times 9 \text{ mm})$  were collected from the Kinango Dam and *C. ferruginea*  $(16 \times$ 9 mm) were collected from the Kadingo River in Kwale District. Miracidia were allowed to hatch in dechlorinated tap water. The snails were individually exposed to 20 miracidia in the wells of a 24-well tissue culture plate for 10, 30 and 60 minutes. After exposure, the snails were removed from wells, and the remaining miracidia were counted under a stereoscopic microscope.

#### RESULTS

Numbers of *B. globosus* and *C. ferruginea* collected monthly in each site are shown in Figure 1. Seasonal changes in total snail population of both species were generally associated with the alternating cycle of rainy and dry seasons. At the end of the long rainy season, snail populations started to increase, reached a peak, and fell during hot dry season. The relative abundance of populations of *B. globosus* and *C. ferruginea* differed by site and year. Therefore, the correlation between annual numbers of *B. globosus* and *C. ferruginea* at each site were analyzed. Inverse correlations were observed between the two snail species. Coefficients of correlation between annual numbers of *B. globosus* and *C.* 

	Number of snails						
Site							
19 21	22	23	24				
58 141	390	41	32				
68 399	561	629	2,212				
13 224	831	149	390				
34 104	353	226	1,480				
68 36	422	106	21				
20 465	366	59	812				
60 127	146	54	41				
31 101	409	201	825				
23 389	700	129	282				
48 106	53	141	573				
58 198	706	200	295				
35 87	65	137	301				
75 104	761	159	191				
21 88	68	46	475				
27 -0.523	-0.650	-0.629	-0.257				
	Site           9         21           58         141           58         399           13         224           34         104           68         36           20         465           60         127           31         101           23         389           48         106           58         198           35         87           75         104           21         88           27         -0.523	Site           9         21         22           58         141         390           58         399         561           13         224         831           34         104         353           58         36         422           20         465         366           60         127         146           31         101         409           23         389         700           48         106         53           58         198         706           35         87         65           75         104         761           21         88         68           27         -0.523         -0.650	Site           9         21         22         23           58         141         390         41           58         399         561         629           13         224         831         149           34         104         353         226           68         36         422         106           20         465         366         59           60         127         146         54           31         101         409         201           23         389         700         129           48         106         53         141           58         198         706         200           35         87         65         137           75         104         761         159           21         88         68         46           27         -0.523         -0.650         -0.629				

 Table 1. Annual numbers of Bulinus globosus and Cleopatra ferruginea, and coefficient of correlation between annual numbers of two species at each site

\* B.g.: Bulinus glogosus, C.f.: Cleopatra ferruginea



Figure 1. Monthly numbers of *Bulinus globosus* and *Cleopatra ferruginea* collected from 7 sites, April 1984 to March 1991.





*ferruginea* varied according to collection sites, but were all negative, ranging from -0.237 to -0.629 (Table 1).

Annual numbers of *C. ferruginea*, *B. globosus* and *S. haematobium* infected *B. globosus* collected at 7 sites are shown in Table 2. In our study area, selective mass-chemotherapy with metrifonate was carried out in February 1984, and that with praziquantel in September 1986, 1988 and 1990 (Sato *et al.*, 1988; unpublished date). Whenever mass-treatment was given, the overall prevalence in our study area fell rapidly after treatment, but rose again after 1 year to the pre-treatment level. Therefore the level of contamination of river in year 3 was comparable to that of years 5 and 7, and the level in

year 4 was comparable to that of year 6. The infection rate of year 3 was lower than those of year 5 and 7, and that of year 4 was lower than that of year 6. Years 3 and 4, when the lower infection rates were recorded, were those when large numbers of *C. ferruginea* and small numbers of *B. globosus* were collected.

The results of the experiments on the relative penetration of miracidia into *B. globosus* and *C. fer-ruginea* are shown in Table 3. Duming the early period of snail-miracidia contact, *B. globosus* was penetrated by a higher number of miracidia than *C. ferruginea* (P < 0.05, Student's *t*-test). Later, however, *C. ferruginea* was also penetrated by a high number of miracidia.

	Number of snails			
Period	Cleopatra Bulinus ferruginea globosus (i		Infected <i>B. globosus</i> (infection rate)	
Year 1 (April 1984—March 1985)	5,073	1,490	71 (4.8%)	
Year 2 (April 1985—March 1986)	2,837	2,892	114 (3.9%)	
Year 3 (April 1986—March 1987)*	3,507	1,028	2 (0.2%)	
Year 4 (April 1987—March 1988)	2,088	930	1 (0.1%)	
Year 5 (April 1988—March 1989)*	1,226	3,466	111 (3.2%)	
Year 6 (April 1989—March 1990)	748	2,794	61 (2.2%)	
Year 7 (April 1990—March 1991)*	835	2,258	25 (1.1%)	

 Table 2.
 Annual numbers of Cleopatra ferruginea, Bulinus globosus, and Schistosoma haematobium infected B. globosus collected from 7 sites

\* The selective mass-chemotherapy with praziquantel was carried out in September 1986, 1988 and 1990.

Table 3. Number (Mean  $\pm$  S.D., N=6) of Schistosoma haematobium miracidia remaining after exposure to Bulinus globosus and Cleopatra ferruginea (Snails were individually exposed to 20 miracidia)

Creation	Minutes of exposure				
Species	10*	30	60		
Bulinus globosus	$10.1 \pm 3.3$	$7.7 {\pm} 2.4$	$7.8 \pm 2.9$		
Cleopatra ferruginea	$14.7\!\pm\!2.7$	$11.8 \pm 4.7$	$9.8{\pm}3.8$		

\* Number of remaining miracidia exposed to two snail species are significantly different at the 0.05 level (Student's *t*-test).

#### DISCUSSION

T. granifera and T. tuberculata, belong to the gastropod family Thiaridae, may be effective competitors with Biomphalaria (reviewed by Pointier and McCullough, 1989). The potential of T. granifera in biological control has been investigated in St. Lucia. In four field trials, B. glabrata was apparently eliminated from marshes and streams 6 to 22 months after the introduction of T. granifera (Prentice, 1983). In Martinique, T. tuberculata was introduced into two groups of watercress beds, and both B. globosus and B. straminae were eliminated from the transmission sites within 3 years of the introduction of the competitor (Pointer et al., 1989). Mkoji et al. (1992) reported that Melanoides tuberculata (Thiaridae) co-exists with Biomphalaria pfeifferi and other pulmonates in Kenyan freshwater habitats, and possibly acts to regulate pulmonate populations. After molluscicide application, all four snail species (M. tuber culata, B. pfeifferi, Lymnaea natalensis and B. globosus) became scarce. Then, B. pfeifferi populations recovered and achieved levels of relative abundance considerably higher than noted prior to molluscicide application; *M. tuberculata* was slow to recover following mollusciciding. In our study area, *C. ferruginea*, also a member of the Thiaridae, was present and was locally abundant. An inverse correlation between populations of *B. globosus* and *C. ferruginea* was observed in a natural setting. The data suggest that *C. ferruginea* has limiting effects on *B. globosus* populations. It is likely that *C. ferruginea* destroys eggs and young snails of the vector and competes with vector snails for space and foods.

In addition to the possible role of C. ferruginea as an effective antagonist to vector snails, the presence of unsusceptible snails to schistosome may reduce the infection rate of snails by acting as a miracidial sponge or as a decoy (Chernin, 1968; Upatham and Sturrock, 1973; Laracuente et al., 1979). Upatham and Sturrock (1973) suggest that unsusceptible snails have a significant effect on diminishing transmission, especially when chemotherapy has been used to reduce the worm burden and egg load in the human population. The experiments on the relative penetration of miracidia into B. globosus and C. ferruginea showed that S. haematobium miracidia also penetrated C. ferruginea. In our study area, the low infection rates in B. globosus were recorded in years 3 and 4 when large numbers of C. ferruginea and small numbers of B. globosus were collected; the infection rate in year 3 was lower than those in year 5 and 7, and the infection rate in year 4 was lower than that in year 6. As was described previously, the level of contamination of water at year 3 and 4 was comparable to that of year 5 and 7, and that of year 6 respectively. Our study may indicate that the predominance of C. ferruginea over B. globosus reduces the infection rate of snails by reducing the number of B. globosus, and, in addition, by reducing the number of miracidia which reach to B. globosus.

*C. ferruginea* is widely distributed in permanent rivers and muddy residual pools in seasonal streams along the east coast of Africa (Kenya, Tanzania, Mozambique and South Africa) where *S. haematobium* is endemic (Brown, 1980). *C. ferruginea* may have a previously unappreciated effect on diminishing the transmission of *S. haematobium* in these coastal habitats. *C. ferruginea* appears to present interesting possibilities as an effective tool for biological control. Further investigations should be attempted to examine this possibility.

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## REFERENCES

- Brown, D. S. (1980): Freshwater Snails of Africa and their Medical Importance. pp. 487. Taylor & Francis, London
- Chernin, E. (1968): Interference with the capacity of Schistosoma mansoni miracidia to infect the molluscan host. J. Parasitol., 54, 509-516
- Laracuente, A., Brown, R. A. and Jobin, W. (1979): Comparison of four species of snail as potential decoys to intercept schistosome miracidia. Am. J. Trop. Med. Hyg., 28, 99-105
- 4) Noda, S., Shimada, M., Sato, K., Ouma, J. H., Thiongo, F. W., Muhoho, N. D., Sato, A. and Aoki, Y. (1988): Effect of mass chemotherapy and piped water on number of *Schistosoma haematobium* and prevalence in *Bulinus globosus* in Kwale, Kenya. Am. J. Trop. Med. Hyg., 38, 487-495
- 5) Mkoji, G. M., Mungai, B. N., Koech, D. K., Hofkin, B. V. and Loker, E. S. (1992): Does the snail *Melanoides tuberculata* have a role in biological control of *Biomphalaria pfeifferi* and other medically important African pulmonates? An. Trop. Med. Parasitol., 86, 201-204

- 6) Pointier, J. P., Guyard, A. and Mosser, A. (1989): Biological control of *Biomphalaria glabrata* and *B. straminea* by the competitor snail *Thiara tuberculata* in a transmission site of schistosomiasis in Martinique, French West Indies. An. Trop. Med. Parasitol., 83, 263 -269
- 7) Pointier, J. P. and McCullough, F. (1989): Biological control of the snail host of *Schistosoma mansoni* in the Caribbean area using *Thiara* spp. Acta Tropica, 46, 147-155
- 8) Prentice, M. A. (1983): Displacement of *Biomphalaria* glabrata by the snail *Thiara granifera* in field habitats in St. Lucia, West Indies. An. Trop. Med. Parasitol., 77, 51-59
- 9) Sato, K., Shimada, M., Noda, S., Muhoho, N. D., Katsumata, T., Sato A. and Aoki Y. (1988): Efficacy of metrifonate in a highly endemic area of urinary schistosomiasis in Kenya. Am. J. Trop. Med. Hyg., 38, 81-85
- Upatham, E. S. and Sturrock R. (1973): Field investigations on the effect of other aquatic animals on the infection of *Biomphalaria glabrata* by *Schistosoma mansoni* miracidia. J. Parasitol., 59, 448-453
- 11) WHO (1984): Report of an informal consultation on research on the biological control of snail intermediate hosts. TDR/VBC-SCH/SI/84.3.