Genetic varation between *Biomphalaria alexandrina* and *Biomphalaria glabrata* snails and their infection with Egyptian strain of *Schistosoma mansoni*

Fayez A. Bakry

Medical Malacology Department, Theodor Bilharz Research Institute, Giza. Egypt

Abstract

Schistosomiasis remains one of the most prevalent parasitic infections and has significant economic and public health consequences in many developing countries. Understanding the host/parasite association is important for control this parasite. Thus, this research aims to learn more about the genetic basis of the snail/parasite relationship to explore novel techniquel for disrupting the transmission of this disease. Therefore, susceptibility of three Egyptian populations of *Biomphalaria alexandrina* (from Damitta, Giza and Fayoum Governorates) and one of *Biomphalaria glabrata* from Germany (Bayer Com.) to an Egyptian strain of *Schistosoma mansoni* from Giza was tested. In addition, genetic variations between these snail populations were determined by PCR technique.

The snail *B. alexandrina* from Damitta and *B. glabrata* were less susceptible to infection with the tested strain of *S.mansoni* than *B. alexandrina* from Giza and Fayoum Governorates. The genetic variations between *B.glabrata* and the Egyptian populations of *B. alexandrina* were obvious. However, no variations were detected between snail populations from Giza and Fayoum. These genetic variations should contribute to a better understanding of the correletions between vectors, parasites and transmission of the disease.

Keywords: *Biomphalaria alexandrina*; *Biomphalaria glabrata*; *Schistosoma mansoni*

1 Introduction

Biomphalaria alexandrina snails play an important role in transmission of *Schistosoma mansoni* in Egypt. Control of schistosomiasis in Egypt was the goal of an ambitious 10-year program coordinated by the Egyptian Ministry of Health and Population and the U.S. Agency for International Development (El-Khoby,1998).

Adaptation of parasites to their local hosts is a common phenomenon, but not universal, and sometimes the pattern is even reversed (Kaltz and Shykoff, 1998). Compatibility between Schistosomes and their snail vectors was found to be related to: (i) the geographic origin of the snail, and (ii) sympatric combinations of snails and parasites produced a greater proportion of compatible infections than allopatric combinations. Schistosomiasis cercarial output of infected snails was found to be related to the geographic origin of the parasite (Manning *et al*., 1995).

Understanding of the genetic basis for schistosome resistance/or susceptibility in the snail vectors is important, because schistosome eradication programs may benefit from the knowledge of the genetic polymorphism of these snails since association between hosts and parasite tend to generate and maintain genetic polymorphism (May, 1985), as has been predicted in models of schistosome-snail interactions (Morand *et al*., 1996).

Several molecular techniques for genetic variability in schistosomiasis snail vectors have been studied (Knight *et al*., 2000; Jannotti-Passos and Souza, 2000; Vidigal *et al*., 2001; Caldeira *et al*.,2001; Souza and Passos, 2001; Tuan and Bortolato, 2001).

Corresponding outher: Fayez A.Bakry; Address: Theodor Bilharz Research Institute, warrak El-Hader,Imbaba,P.O.30;E.mail:Fayezbakery@yahoo.com.

Many attempts have been made to define markers for susceptibility/resistance in either *Biomphalaria glabrata* (Sire *et al*., 1999; Knight *et al*., 1999) or *Biomphalaria tenagophila* (Abdel-Hamid *et al*., 1999). Most attempts involved linkage analysis by examining pigmentation and allozyme phenotypes in resistant and susceptible snails. To date, no single association between such markers and linkage to the resistant phenotype has been identified in these snail species.

Recently, several investigators showed that random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is useful for distinguishing between and within different *Biomphalaria* snail species (Abdel-Hamid *et al*., 1999; Knight *et al*., 1999; Kristensen *et al*., 1999; Sire *et al*., 1999).

Due to the high applicability of RAPD-PCR and the quality of the results obtained, this technique have been used to investigate the intra- and inter-populational variability infection to detect genetic markers associated with resistance/or susceptiblity of *B. glabrata* and *B. tenagophila* snails to *S.mansoni* infection (Oliveira *et al*., 2008).

The present study aims to study susceptibility of *Biomphalaria* snails to infection with an Egyptian strain of *S. mansoni* and also, to determine the genetic variability between Egyption populations of *B. alexandrina* and *B.glabrata* (from Bayer Com.) using PCR technquie.This research will help to learn more about tha genetic varations between *Biomphalaria* species with the hope of finding novel ways to disrupt the transmission of this disease.

2 Material and methods

Biological materials:

Biomphalaria alexandrina snails were collected from the irrigation canals at Giza, Fayoum and Damietta Governorates (Egypt) and maintained at Medical malacology laboratory in Theodor Bilharz Reseach Institute (TBRI) under suitable conditions. In addition, *B. glabrata* snails laboratory stock from Bayer Company (Germany) were included in this study. *S.mansoni* miracidia of the Egyptian strain(from Giza) were obtained from Schistosome Biological Supply Centre (SBSC), TBRI, Egypt.

Snails' infection:

Four identical groups (5-7mm) of *Biomphalaria* spp *(*25 snails/ group), the first group was *B. glabrata,* the 2nd, 3rd and 4th groups were *B. alexandina* from Giza, Fayoum and Damietta, respectively. Five replicates were prepared for each group.The snails were individually exposed to miracidia (10 miracidia/ snail) for 24 hours at 24°C and ceiling illumination.

After exposure, snails from each group were collected, washed and transferred to clean aquaria with dechlorinated water. They were daily fed boiled lettuce leaves. Dead snails were daily removed from the aquaria and the mortality rate was calculated. After three weeks post miracidial exposure; surviving snails were individually examined for cercarial shedding in multidishes under artificial light for 3 hours. The produced cercarial suspension was poured in a graduated Petri-dish and all cercariae were counted after adding few drops of Bouin’s fluid using a dissecting microscope. Infected snails were isolated and kept in special aquaria in complete darkness and examined for cercarial production twice weekly (3hours each) till all snails dead. Prepatent period, total cercarial production/snail and duration of cercarial shedding were calculated.

Molecular analysis:

At least six snails per population (*B. glabrata* and *B. alexandrina* from three Egyptian Governorates) were singled out for molecular study. Snails were preserved in absolute ethanol (96-100%) at 4°C. The nuclear first internal transcribed spacer (ITS1) and 5.8S-AS (ITS2) sequences (GenBank U65223) for *Biomphalaria* spp (Eurofins MWG GmbH) were reported by Lotfy *et al* 2004.

DNA was extracted from the tip of the head foot region of snails individually, the isolation of genomic DNA from small size of tissues by the peqGOLD MicroSpin Tissue DNA Kit (PeqLab Biotechinologie GmbH Erlangen,Germany) ,this method used by Anja *et al*.,2008. DNA integrity and concentration were estimated by comparison with molecular weight standard on 1.5 agarose gel electrophoresis.

DNA amplification by PCR

The genotypes of the both *Biomphalaria* snails species were determined using primers which complemented conserved regions in the 3' end of the 18S gene (ITS1-S, 5' CCATGAACGAGGAATTCCCAG 3') in combination with 5.8S-AS (ITS2- 5' TTAGCAAACCGACCCTCAGAC 3'), by PCR as in the method of Simpson *et al*. (1993), with some modifications.

Genomic DNA was amplified with a Thermal Cycler (MJ Research MiniCycler PTC-150, Canada) using PCR. Each reaction was carried out in a final volume of 25 µL containing 12 µL of marker PCR, 3 µL of prime ITS1-S, 3 µL of prime ITS2, 2 µL of distal water and 5 µL of DNA of snails and 2.5 unit Taq DNA polymerase was prepared. The amplification conditions were as follows: 1 cycle at 95°C for 15 min, 2 cycles at 94°C for 1min,3 cycles at 59.8 °C for 1 min, 4 cycles at 72°C for 1 min, 5 cycles at 72°C for 10 min and 6 cycles for 40°C for 24 hours and 36 cycles during which the annealing temperature was changed to 40°C and the time of the extension step was increased to 5 min during the final cycle. As control, PCR was run without DNA genome. After DNA thermal cycle, PCR products were analyzed by using 1.5 agarose gel electrophoresis (electrophoresis power supplies E455) and silver stained to resolve amplified fragments as described by Santos *et al*. (1993).

All PCR reactions and other molecular analyses, and snail infection were carried out in the institute for Zoomorphologie, Zellbiologie and Parasitologie, Heinrich-Heine University, Dusseldorf, Germany.

3 Results

The results (Fig.1) indicated that after 25 days from exposure to miracidia the survival rates of *B. glabrata* and *B. alexandrina* from Damietti (88% and 80% respectively) were significantly higher than to of *B.alexandrina* Fayoum and Giza (64% and 72%, respectively, $P<0.001$).

Susceptibility of Egyptian *Biomphalaria* snails and *B. glabrata* to *S. mansoni* infection was studied starting 25 days post-infection. The results (Fig.1) pointed out that infection rates of *B.glabrata* and *B. alexandrina* from Damietta 8% and 16%,respectively) were significantly less than those of *B. alexandrina* from Fayoum and Giza (56% and 64%, respectively,$P<0.001$). The prepatent (Incubation) period (Fig.2) of *Schistosoma* in *B. alexandrina* from Giza and Fayoum was significantly ($p<0.05$) shorter (28 and 34 days, respectively) than in *B.alexandrina* from Damietta and *B.glabrata* snails (38 and 44 days, respectively). The mean number of periodic cercarial production per snail (Fig.3) of infected *B. alexandrina* from Giza (230 cercariae per snail) was higher than that of infected *B. glabrata* snails (33 cercariae per snail). The same phenomenon was recorded for the mean duration of cercarial shedding for *B. alexandrina* from Giza as it

was significantly ($p<0.001$) longer (9.4 days) than that of *B. glabrata* and *B. alexandrina* snails from Damietta (3.5 and 4.2 days, respectively.

It is concluted that *B.glabrata* from Bayer strain (Germany) and *B.alexandrina* snails from Damietta (Egypt) were less susceptibile to infection with an Egyptian strain of *S. mansoni* (Giza) than *B.alexandrina* from Fayoum and Giza Egypt).

Regarding to the genomic DNA amplified with primer 1, 2, the results showed a polymorphic band of nearly 640 bp in *B. alexandrina* from Damietta (Fig.4), while that of the same snail species from Fayoum and Giza exhibited 501 bp. However, a higher bp was recorded for *B. glabrata* snails, 1080 bp. The results indicated that there is no genetic varations between *B.alexandrna* from Fayoum and Giza. Although, the specimens from Damietta within the same species showed few differences. There are more genetic varations between *B.glabrata* (Bayer strain) and Egyption populations of *B. alexandrina*.

4 Discussion

The present results showed that *B. glabrata* and *B. alexandrina* snails from Damietta were less susceptible to infection with the Giza Egyptian strain of *S. Mansoni* than *B. alexandrina* snails from Giza and Fayoum. This agrees with the previous findings of Yousif *et al*. (1998), on susceptibility of *B. alexandrina, B. glabrata* and a hybrid snail of both, from natural habitats in Egypt, to infection with six human Egyptian strains of *S. mansoni* and a laboratory strain of human origin. They found that the infection rate of all Egyptian *S. mansoni* strains was significantly higher in *B. alexandrina* than each of *B. glabrata* and the hybrid snail. Moreover, Manning *et al*.(1995) carried out cross-infection experiments with *Bulinus* snails of two geographic strains and exposing them to parasites of both geographic strains and suggesting the existence of geographic compatibility among natural populations of snails and trematodes. The same results were concluded by Fernandez and Thiengo (2006) using *Biomphalaria amazonica* and *Biomphalaria occidentalis* from Manso Dam, Mato Grosso, Brazil and infected them with three strains of *S. mansoni.* Lively (1989) indicated a strong evidence for local adaptation by the parasite and there is a genetic basis to the host-parasite interaction.

The dynamic interaction between molluscs and their trematode parasites leads either to a state of co-existence, in which the trematode thrives and produces subsequent stages of its life-cycle, or to incompatibility, where the trematode is either destroyed and eliminated by the host snail defensive responses or fails to develop because the host is physiologically unsuitable (Van der Knaap and Locker, 1990).

In the present study *B .glabrata* and *B. alexandrina* from Damietta have significantly longer incubation (prepatent) period and duration of cercarial production than those of *B. alexandrina* snails from Fayoum and Giza. This result agrees with Yousif *et a*l (1996) who showed that *B. glabrata* has significantly longer incubation period and duration of cercarial production than *B. alexandrina* when exposed to laboratory strain of *S. mansoni* (SBSC,TBRI) from Egypt.

The cercarial production of *B. glabrata* and *B.alexandrina from* Damietta was significantly lower than that of *B.* alexandrina from Fayoum and Giza. This is in parallel with that of Frandsen (1979) who found that *B. glabrata* infected with various strains of *S.mansoni* from St. lucia, the West Indies produced variable amounts of cercariae. Moreover, Yousif *et al*. (1998) found that each of B.*glabrata, B. alexandrina* and their hybride snails had different rates of infection and different values of total periodic cercarial production with various strains of *S. mansoni*.

The results indicated no genetic varations between *B.alexandrna* from Fayoum and Giza were recorded. Howevers, the specimens from Damietta within the same species showed few differences.Moreover, more genetic varations between *B.glabrata* (Bayer Com.Germany) and Egyption populations of *B. alexandrina* were seen. These results support the data of Oliveira *et al*. (2008) on genetic variability among susceptible

and resistant strains within and between *B. glabrata* and *B. tenagophila* using RAPD-PCR. They indicated great genetic variations within the two snail species using three different primers (intrapopulational variations), while specimens from the same snail species showed few individual differences between the susceptible and resistant strains. Several investigators showed that random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is useful for distinguishing between and within different *Biomphalaria* snail species (Abdel-Hamid *et al*., 1999; and Sire *et al*., 1999).

The extremely low amount of variation among the *B. alexandrina* sequences suggests that this species may have had a restricted geographic range in the past and may now be expanding its range. However, because the sequence variability is so low, there is very little phylogeographic signal to indicate the direction of *B. alexandrina* expansion (Fernandez and Thiengo, 2006).

Similarily, Davies *et al*. (1999) compared the genetic population structures of the freshwater snail *Bulinus globosus* and its trematode parasite *Schistosoma haematobium* from 8 river sites in the Zimbabwean highveld using randomly amplified DNA markers. There was significant variability between snail populations collected at different sites, but schistosome populations only showed differentiation at a wider geographical scale (between 2 non-connected river systems). For snails, genetic distance was better correlated with proximity along rivers than absolute geographical separation. In contrast, schistosome genetic distance was better correlated with absolute geographical separation than proximity along rivers.

species-specific PCR assays and of ITS1 and ND1 sequencing data show that *Biomphalaria* snails collected from some Egyptian water courses were *B. alexandrina* and no evidence for the presence of *B. glabrata* or hybrids of *B. alexandrina* with *B. glabrata* (Lotfy *et al*., 2004), even though 19 of these field Egyptian samples came from the Nile Delta or nearby, where both *B. glabrata* and hybrids had been previously reported(Yousif *et al*.,1996&1998)

The delay in development of the parasite within some snail strains was declored by Richords and merit (1972) and Richords and Shade (1987) on the baseis of susceptibility or resistance of the snails to *S. mansoni* was dependent on host genetic background and/or on the interaction between the gene products of the mollusk and the parasite.

In the present study, the identification of polymorphic bands was based on comparison of the band patterns on the same gel for the two snail species and those detected in all individuals of the same strain according to Larson *et al*. (1996) technique.

The result indicated that there are more genetic varations between *B.glabrata* (Bayer.com, Germany) and Egyptian populations of *B. alexandrina,* meanwhile the specimens from Damietta within the Egyptian species showed few individual differences. These genetic varation could be responsible for low susceptibility of these snails to infection with an Egyptian strain of S. mansoni and should contribute to a better understanding of the correlations between vectors, parasites and transmission of the disease.

This finding supports the results of Rizk and Abdel-Hamid (2001) that the genetics of the vector snails play a major role in determining various outcomes of the parasite. The dynamic interaction between molluscs and their trematode parasites leads either to a state of co-existence, in which the trematode thrives and produces subsequent stages of its life-cycle, or to incompatibility, where the trematode is either destroyed and eliminated by the host snail defensive responses or fails to develop because the host is physiologically unsuitable (Van der Knaap and Locker, 1990).

This approach has indeed attempted to add more to our knowledge about the genetic diversity of both snail species in the field and their roles in transmission of schistosomiasis in different localities in Egypt. It is anticipated that the development of molecular markers associated with resistant/susceptible phenotypes should lead to a thorough strategy for the control of schistosomiasis disease.

ACKNOWLEDGMENTS

This research was supported by the instit□t für Zoomorphologie, Zellbiologie a Parasitologie Heinrich-Heine-Universit□t Düsseldorf. The author is grateful to Prof Dr. Heinz Mehlhom and Prof Dr. DHaese for continuous advice, discussions and critical suggestions.

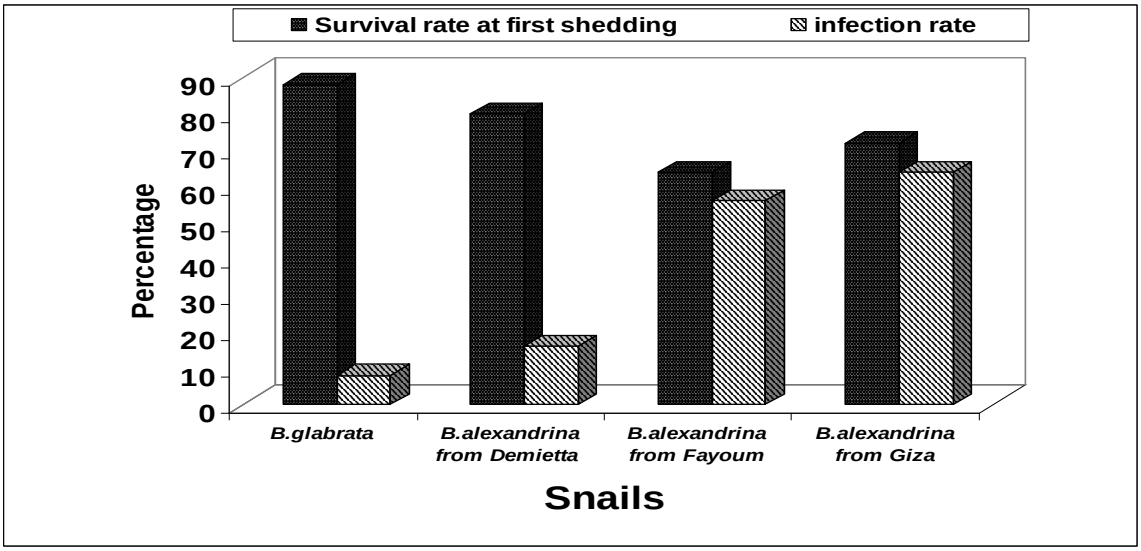

Fig (1). Survival and Infection rates of *B . glabrata* (Bayer Com.) and *B. alexandrina* snails from three Egyptian Governorates (Damietta, Giza and Fayoum) post exposure to miracidia of S.mansoni Egyptian strain.

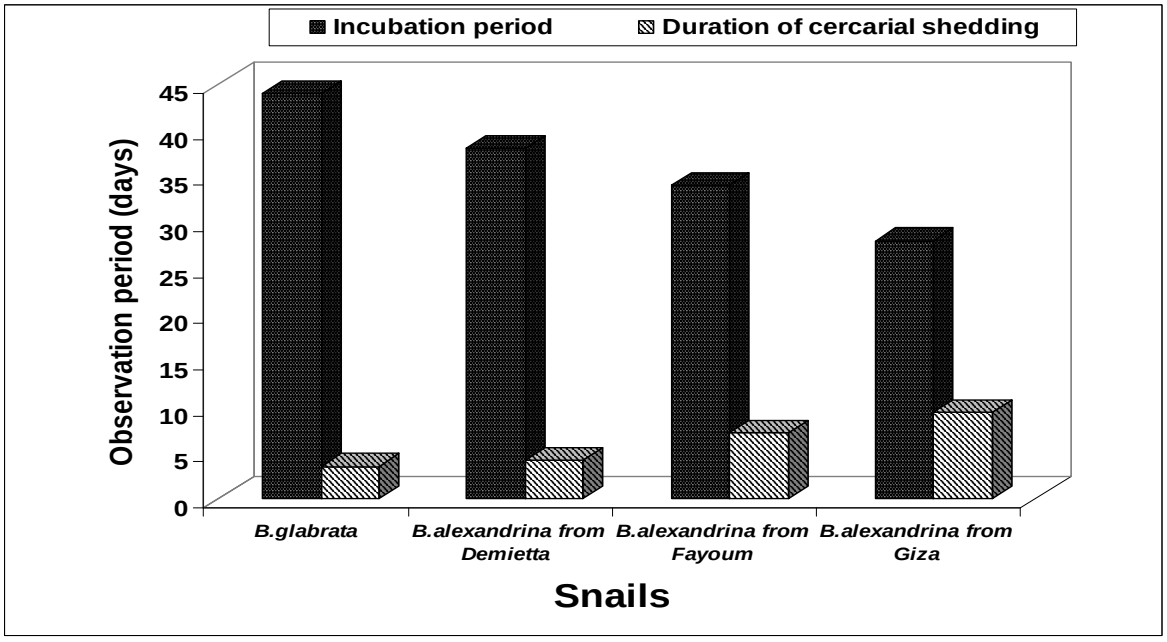

Fig (2) Incubatiom period and Duration of cercarial shedding of *B . glabrata* (Bayer Com.) and *B. alexandrina* snails from three Egyptian Governorates infected with an Egyptian strain of *S. mansoni*.

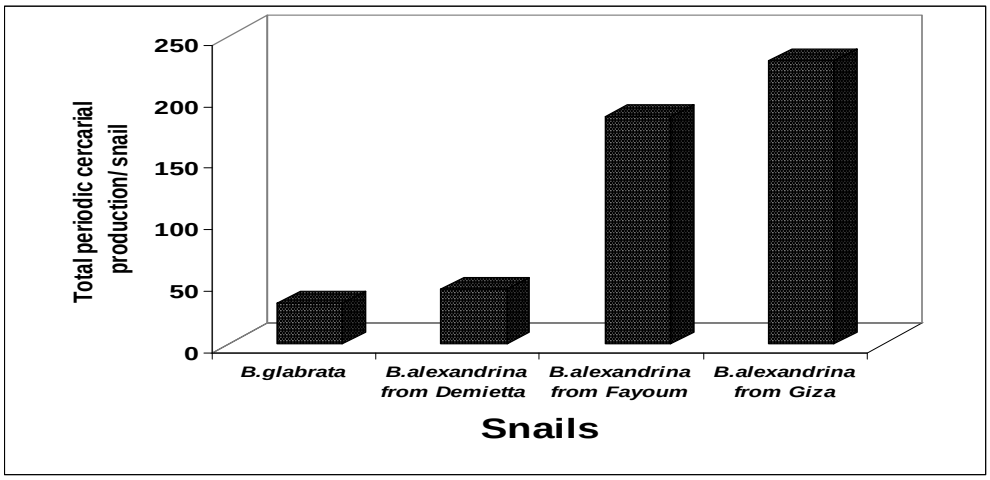

Fig (2) Total periodic cercarial production/snail for *B. glabrata* (Bayer Com.) and *B. alexandrina* snails from three Egyptian Governorates infected with an Egyptian strain of *S. mansoni*.

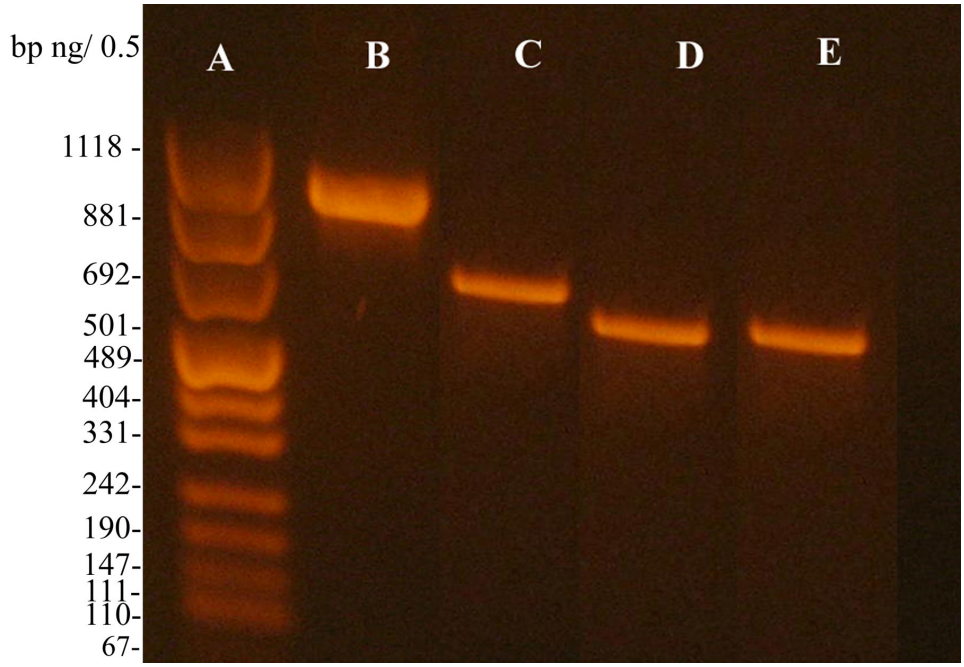

Fig (4). Genetic variability among Egyptian populations of *B. alexandrina* and *B.glabrata* (Bayer.Com) evaluated by analyzing the electrophoretic band patterns obtained on the gels. A=Marker (0.5µg/lane,8cm length gel, 1xTBE,5V/CM,1.5h), B= *B.glabrata* (Bayer. Com.) C= *B.alexandrina* from Damietta, D= *B.alexandrina* from Fayoum, E= *B.alexandina* from Giza.

REFERENCES

1. Abdel-Hamid, A.H., de Molfetta, J.B., Fernandez, V., Rodrigues, V., 1999. Genetic variation between susceptible and non-susceptible snails to *Schistosoma* infection using random amplified polymorphic DNA analysis (RAPDs). Rev Inst Med Trop São Paulo, 41: 291-295.

2. Anja, L., Anne, B., Michael, G., 2008; Cultivation of human bone marrow stromal cells on three-dimensional scaffolds of mineralized collagen: influence of seeding density on colonization, proliferation and osteogenic differentiation. J Tiss Eng Reg Med, (2): 400-407.

3. Caldeira, R.L., Vidigal, T.H., Simpson, A.J., 2001. Carvalho OS. Genetic variability in Brazilian populations of *Biomphalaria straminea* complex detected by simple sequence repeat anchored polymerase chain reaction amplification. Mem. Inst Oswaldo Cruz, 96: 535-544.

4. Davies, C.M., Webster, J.P., Kruger, O., *et al*.**,** 1999 Host-parasite population genetics: A cross-sectional comparison of *Bulinus globosus* and *Schistosoma haematobium.* Parasitolology, 119: 295-302.

5. El-Khoby, T., Galal, N., Fenwick, A., 1998. The USAID Government of Egypt's Schistosomiasis Research Project (SRP). Parasitol Today, 14: 92–96.

6.Fernandez, M.A., Thiengo, S.C., 2006. Susceptibility of *Biomphalaria amazonica* and *Biomphalaria occidentalis* from Manso Dam, Mato Grosso, Brazil to infection with three strains of *Schistosoma mans*oni Mem. Inst. Oswaldo Cruz 101: 235-237.

7. Frandsen, F., Madsen, H., 1979 A review of *Helisoma duryi* in biological control. Acta Tropica, 63*:* 67–84.

8. Jannotti-Passos, L.K., de Souza, C.P., 2000. Susceptibility of *Biomphalaria tenagophila* and *Biomphalaria straminea* to *Schistosoma mansoni* infection detected by low stringency polymerase chain reaction. Rev. Inst Med Trop São Paulo 42: 291-294.

9. Kaltz, O., Shykoff , J.A., 1998. Local adaptation in host–parasite systems**.** Heredity, 81: 361–370.

10. Knight, M., Miller, A.N., Patterson, C.N., Rowe, C.G., *et al*., 1999. The identification of markers segregating with resistance to *Schistosoma mansoni* infection in the snail *Biomphalaria glabrata*. Proc. Natl Acad Sci U S A, 96: 1510-1515.

11.Knight, M., Ongele, E., Lewis, F.A., 2000. Molecular studies of *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Int J Parasitol , 30: 535-541.

12.Kristensen, T.K., Yousif, F., Raahauge, P., 1999. Molecular characterisation of *Biomphalaria* sp in Egypt J Mollus Stud, 65: 133-136.

13. Larson, S.E., Anderson, P.L., Miller, A.N., Cousin, C.E., *et al*., 1996. Use of RAPD-PCR to differentiate genetically defined lines of an intermediate host of *Schistosoma mansoni, Biomphalaria glabrata*. J Parasitol, 82: 237-244.

14. Lively, C.M., 1989. Adaptation by parasitic trematode to local populations of its snail host. Evolution, 43 (8): 1663-1671.
15. Lotfy, W.M., DeJong, R.J., Black, B.S., Loker, E.S., 2004. Specific identification of Egyptian *Biomphalaria* species and possible hybrids using the polymerase chain reaction based on nuclear and mitochondrial loci. Mol Cell Probes, 19: 21–25.
16. Manning, S.D., 1995 Woolhouse ME, Ndamba J. Geographic compatibility of the freshwater snail *Bulinus globosus* and schistosomes from the Zimbabwe Highveld. Int J Parasitol, 25: 37-42.
17. May, R.M., 1985. Host-parasite associations: their population biology and population genetics. In: Ecology and genetics of host-parasite interactions (Rollinson D and Anderson RM, eds.). Academic Press, London, 243-262.
18. Morand, S., Manning, S.D., 1996. Woolhouse ME. Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility. Proc Biol Sci, 263: 119-128.
19. Oliveira**,** A.L.D., Silva, D.D.A., 2008. Zanotti-Magalhaes EM, *et al*. Schistosome/mollusk: Genetic compatibility. Mol Res, 7: 518-526..
20. Richards, C.S., Merritt, J.W., 1972. Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni* infection. Am J Trop Med Hyg , 21: 425-434.
21. Richards, C.S., Shade, P.C., 1987. The genetic variation of compatibility in *Biomphalaria glabrata* and *Schistosoma mansoni*. J Parasitol, 73: 1146-1151.
22. Rizk, M. Z., Abdel-Hamid, Z.A. 2001. Genetic basis for schistosome resistance in the intermediate hosts: An approach for biological control of schistosomiasis. The 7th International Symposium on Schistosomiasis, Ruprecht-Karls-Universität, Heidelberg,, 18th - 21st October.
23. Santos, F.R., Pena, S.D., Epplen, J.T., 1993. Genetic and population study of a Y-linked tetranucleotide repeat DNA polymorphism with a simple non-isotopic technique. Hum. Genet, 90: 655-656.
24. Simpson, A.J.G., Dias Neto, E., Steindel, M., Caballero OLSD, et al., 1993. The use of RAPDs for the analysis of parasites. In: DNA Fingerprinting: State of the Science (Pena SDJ, Chakraborty R, Epplen JT and Jeffreys AJ, eds.). Birkhauser, Verlag Basel, 331-337.
25. Sire, C., Durand, P., Pointier, J.P., Theron, A., 1999 Genetic diversity and recruitment pattern of *Schistosoma mansoni* in a *Biomphalaria glabrata* snail population: a field study using random-amplified polymorphic DNA markers. J Parasitol, 85: 436-441.
26. Souza, C.P., Passos, L.K., 2001. Resistance of *Biomphalaria occidentalis* from Varzea das Flores dam, Minas Gerais, to *Schistosoma mansoni* infection detected by low stringency polymerase chain reaction. Mem Inst Oswaldo Cruz, 96: 381-383.

27. Tuan, R., Bortolato, P.C., 2001. Genetic markers from *Biomphalaria tenagophila* (Gastropoda: Pulmonata: Planorbidae) obtained by the double stringency polymerase chain reaction technique. Mem Inst Oswaldo Cruz 2001, 96: 435-436.
28.Van der Knaap, W.P.W., Locker, E.S., 1990. Immune mechanisms in trematode-snail interactions. Parasitology, 6: 181-187.
29.Vidigal THD,A., Caldeira, R.L., Simpson, A.J.G., Carvalho, O.S., 2001. Identification of *Biomphalaria havanensis* and *Biomphalaria obstructa* populations from Cuba using polymerase chain reaction and restriction fragment length polymorphism of the ribosomal RNA intergenic spacer. Mem Inst Oswaldo Cruz, 96: 661-665.
30. Yousif, F., Haroun, N., Ibrahim, A., El-Bardicy, S., 1996. *Biomphalaria glabrata*: a new threat for schistosomiasis transmission in Egypt. J Egypt Soc Parasitol; *26:* 191–205
31. Yousif, F., Ibrahim, A., Abdel-Kader, A., El-Bardicy, S., 1998. Invasion of the Nile Valley in Egypt by a hybrid of *Biomphalaria glabrata* and *Biomphalaria alexandrina*, snail vectors of *Schistosoma mansoni*. J Egypt Soc Parasitol, *28:* 569–582.