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Anatomy and Parameters of Mating System in *Bulinus globosus* (Morelet, 1866) a Species of Medical Interest in Ivory Coast

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***Abstract*—** *Reprocutive strategies in the freshwater hermaphroditic snail Bulinus globosus, a common intermediate host of schistosomiasis in Ivory Coast has been studied. The study was carried out from 50 wild individuals, and 100 first generation offspring young snails where chosen and separated into two groups of 50 snails. One group was set aside for self-fertilization and snails were kept isolated, the other group was designed for cross-fertilization. The two groups were compared for individual's size, weight and subsequently monitored for the number of eggs laid, and the survival of young snails. Species identity was confirmed by conchiliogical and anatomical methods. So the test between cross-fertilization population and autocrossing populations shows a low positive waiting time of 4.74 days and maximum inbreeding depression. This study confirms that Bulinus globosus is an outcrosser that can self-fertilize depending on the availability of a sexual partner and ecological conditions. This characteristic may be an advantage in colonizing new habitats.*

***Keywords*—** *Bulinus globosus, reproductive strategies, waiting time, Inbreeding depression, Ivory Coast.*

I. INTRODUCTION

Snails are subject to very unstable environments, so they must adopt life strategies that guarantee the survival of their species. In freshwater molluscs, about 40% of genera are hermaphrodites; almost two-thirds of the species, most of them are gastropods. This is the case of *Bulinus globosus* which is a species found in most African freshwaters (Brown, 1994). The hermaphrodite organisms are an example of the evolution of the strategies of reproduction (Alonso, 2006) and are defined in biology as organisms that possess a male and female functional reproductive system for at least part of their life. The evolution of reproductive systems is the result of selection pressures and environmental constraints. The interaction between the two can help to understand the maintenance of mixed reproduction systems (Tsitrone, 2001). self-fertilization, cross-fertilization, or a mixed system is an evolutionary dilemma (Henry, 2002). Depending on the ecological factors of the environment, some hermaphrodites may reproduce by self-fertilization or cross-fertilization. They also play an important role in transmission of human and animal parasitic diseases (Ndiaga and Anis, 2010). This is the case of species of the genus *Bulinus* belonging to the family Planorbidae. *B. globosus* is one of the species representing this group with a medical interest in several areas of Côte d'Ivoire (Cecchi et al., 2007). Indeed this species is the main intermediate host of *Schistosoma haematobium*, parasite of urinary schistosomiasis. Control of vectors is an essential aspect of disease control parasites transmitted by them. A better understanding of the functioning of these vectors across the life story traits will obviously help fight effectively against parasitosis. *B. globosus* is an outcrosser that can self-fertilize when isolate before any copulation as occurred (Jarne *et al*., 1991). Also, several parameters of mating system are not available concerning the life history traits of this important species (Escobar *et al*., 2011). In this study, the anatomy of *B. globosus* is presented and then the age at first reproduction and the waiting time are also determined.

II. MATERIALS AND METHODS

A. Anatomical Study

Specimens of *B. globosus* were collected in a fish pond in Daloa city (6°52'38.5"N; 6°28'53.6"W) using a long-handled sieve (mesh size 1 mm) and transferred to the laboratory alive. The adult specimens were allowed to relax overnight using menthol. They were then immersed for 40 seconds in water heated at 70 °C, from which they were transferred to water at room temperature. Soft parts were drawn from the shell using a small forceps and fixed in slightly modified Railliet-Henry fluid (distilled water 930 ml, sodium chloride 6 g, formalin 50 ml, glacial acetic acid 20 ml). Shell width and height were measured to the nearest 0.1 mm using calipers. The larger snails (up to 25 specimens) were dissected under stereomicroscope for morphological and anatomical study.

B. Data Collection

Apparent Inbreeding Depression (AID) and the Waiting Time (WT) in *B. globosus* were estimated using a standard protocol, defined by Tsitrone *et al.* (2003) and Escobar *et al.* (2007, 2011). Thus, 50 wild individuals ranging in size from 11 to 22 mm and with a mass of between 0.1681 and 0.5920 g were sampled in a fish pond in Daloa city (6°52'38.5"N; 6°28'53.6"W) and brought alive to the laboratory where they were placed in aquariums at 25 °C under a 12-h light: 12-h dark photoperiod and fed ad libitum with boiled lettuce twice a week. Water was changed weekly. At the beginning of experiments, G0 individuals were maintained in high-density conditions (~10 snails per litter) for one week to ensure that individuals had access to partners, thus maximizing copulation opportunities. After this period, G0 individuals were isolated in 200-mL plastic boxes (Fig. 1). Eggs from 20 G0 snails were isolated and the offspring (100 G1 individuals) were reared

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under optimal conditions of food and water. These were divided into boxes of 200 mL at a rate of 5 individuals per box and taking care to identify individuals from the same family (having the same parent) and not to mix the families. After 15 days, when the newborns reach the size of 3 to 5 mm, they were separated and individually isolated in 100 boxes of 200 mL. G1 snails were split into two treatments: (1) obligate self-fertilization: 50 virgin individuals were isolated throughout their lives, never encountering mates; and (2) facultative cross-fertilization: 50 other individuals were isolated most of the time, but encountered multiple adult partners (4 to 5 individuals of the same treatments) 3 times a week for 6h per mating session (i.e., 18h/week); mates were distinguished by a harmless dot of paint on the shell (Henry and Jarne 2007). These regular exposures must begin before sexual maturity characterized by the onset of spawning finally highlights the influence of partners and determine the waiting time. The experiment was conducted for 23 weeks.

The dataset includes Apparent Inbreeding Depression (*AID* and the waiting time (*WT*).

In both treatments, we measured the age at first reproduction (T) of G1 individuals by checking for the presence of egg capsules every 2–3 days and estimated the waiting time as $WT = T_S - T_O$, where T_S and T_O, are mean ages at first reproduction in the obligate self-fertilization (*S*) and the facultative cross-fertilization (*O*) treatments. After egg-laying, live (free-moving) individuals were counted. Juvenile survival (*W*) of G2 snails was calculated in each treatment as the number of living hatchlings divided by the number of eggs laid. Subsequently, the "apparent inbreeding depression" on juvenile survival was estimated as $AID = 1 - W_S / W_O$, where W_S and W_O stand for the mean juvenile survival of offspring from the obligate self-fertilization and the facultative cross-fertilization treatments, respectively.

C. Data Analysis

The Kruskal-Wallis and Mann-Whitney non-parametric tests were used to determine differences in the parameters measured. Analyzes were performed using the XLSTAT *7.5.2* software in Excel.

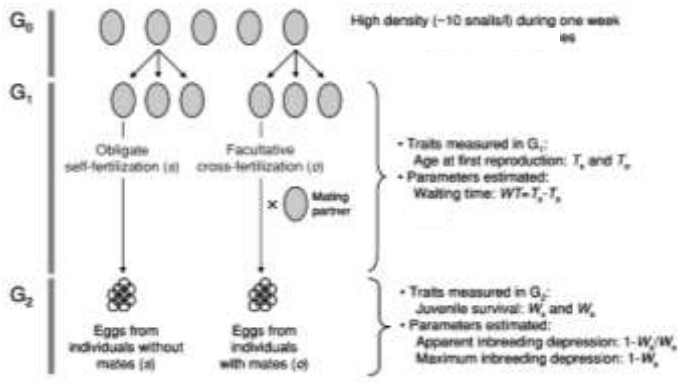

Fig. 1. Schematic diagram of the experimental protocol used to estimate the waiting time and apparent inbreeding depression in *B. globosus*. Filled ovals refer to individuals. G_i refers to generations (Escobar *et al.*, 2011).

III. Results and Discussion

A. Chonciliology and Anatomy

Shell of specimens recorded is globose, sinister, smooth, moderately lustrous and translucent. The spire is short and suture slightly impressed with fine close-set lines of growth (Fig. 2). Whorls 5 is very large, about ¾ total length of shell and regularly and rapidly increasing.

Fig. 2. Shells of *Bulinus globosus* from Ivory Coast, ventral and dorsal view (Photo, J. P. Pointier).

The reproductive system of *B. globosus* consists of an ovotestis with few follicles, a seminal vesicle and a short ovispermiducte. The prostate includes many diverticula divided into two branches and starting from the same point. The long vas deferens follows the penis sheath at least twice shorter and narrower than the prepuce. The vagina is short, the uterus wide and the large spermatheca is connected by a long duct (Fig. 3)

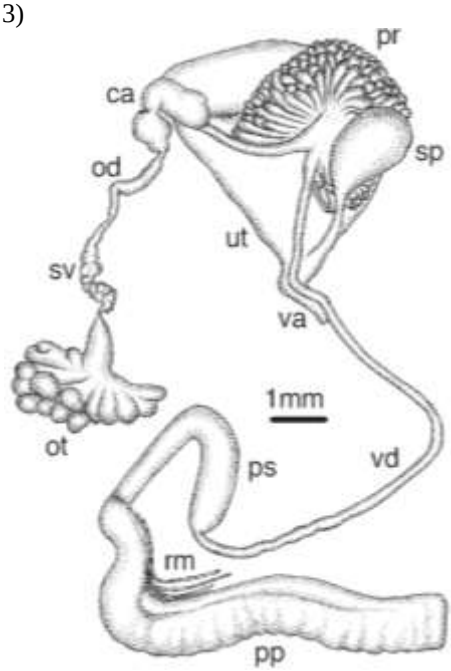

Fig. 3. Anatomy of the reproductive system of *Bulinus globosus* from Ivory Coast: ca carrefour, ot ovotestis, od ovispermiduct, sv seminal vesicles, rm muscular retractor, pr prostate, pp preputium, ps penis sheath, sp spermatheca, va vagina, vd vas deferens, ut uterus; (Bony and Pointier).

B. Mating Parameter

The mean size at first reproduction is slightly higher in cross-fertilization (4.89 mm) than in self-fertilization (4.63 mm), but this difference is not significant (Table I).

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Individuals in cross-fertilized reproduced 37.82 days after birth, while self-fertilization 42.56 days after birth. This difference of 4.74 days, called waiting time, is not significant (P = 0.176).

B. globosus had a positive waiting time, which confirms that it is an outcrosser. This waiting time is higher than the values displayed according to Escobar *et al.* (2011) by the planorbid snails *Biomphalaria glabrata* (3.5 ± 4.5 days), *Indoplanorbis exustus* (1.7 ± 4.2 days) and lower hat those of *Helisoma anceps* (44.4 ± 11.0 days), *H. duryi* (60.3 ± 43.5 days), *H. trivolvis* (33.2 ± 18.3 days) and *Planorbula armigera* (62.9 ± 7.6 days).

The survival rate of juveniles is better for outcrosser G2 offspring at a rate of 0.63 ± 0.27 while that of self-fertilizers is 0.57 ± 0.31, and the Apparent Inbreeding Depression estimated in *B. globosus* was 0.09 ± 0.23. These results are in accordance with values obtained by Njiokou *et al.* (1994).

The study in the outcrosser *P. acuta* highlighted that the age at first reproduction and the survival rate of the self-fertilized offspring are the main factors influencing the waiting time and inbreeding depression, respectively (Tsitrone *et al*. 2003; Escobar *et al.* 2007; Escobar *et al.* 2009). Here, we found that *B. globosus* displayed a relative low waiting time and ID_{max}, this pattern predicts that this species although outcrosser can self-fertilized if isolated.

TABLE I. Parameters of the mating system in *Bulinus globosus*. *Z*= mean size at first reproduction; *T*= mean age at first reproduction; *WT*= Waiting Time; *RWT*= Relative waiting Time; *W*= Juvenile Survival; *AID*= Apparent Inbreeding Depression; ID_{max}= Maximum Inbreeding Depression.

	Outcrossed (*o*)	**Selfed (*s*)**
Z (mm)	4.89 ± 0.63	4.63 ± 0.52
T (days)	37.82 ± 3.6	42.56 ± 4.6
WT (days)	4.74 ± 0.21	
***W* (G2 ind.)**	0.63 ± 0.27	0.57 ± 0.31
***AID* (1-Ws/Wo)**	0.09 ± 0.23	
ID_{max}	0.43	

IV. Conclusion

In conclusion, the shell of *Bulinus globosus* from Ivory Coast is globose, sinister, smooth with a short spire and its reproductive system is typical of Bulininae. *B globosus* observed a waiting time of 4.74 days before reproducing by self-fertilization in the absence of a sexual partner. So, it is an outcrosser that can self-fertilized if isolated. this represents a selective advantage in the colonization of different habitats.

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