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Short Communication

# Mating Behaviour in Two *Schistosoma mansoni* Intermediate Hosts (*Biomphalaria pfeifferi* and *Biomphalaria camerunensis*) in Cameroon

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**Abstract**

*Biomphalaria pfeifferi* and *B. camerunensis* are *Schistosoma mansoni* intermediate hosts. The knowledge of their mating system and mating behaviour is of high interest in mastering the transmission and evolution of a potential resistant gene introduced in a population. This study was therefore conducted to provide further knowledge on the mating behaviour of these two *S. mansoni* intermediate hosts in Cameroon. Mating behaviour was observed in three populations, one of *B. pfeifferi* and two of *B. camerunensis*. Mature virgin young snails (G1) were paired and followed up daily, from 8 am to 5 pm, during three consecutive days. The courtship behaviour, the number of copulations, as well as the sexual role assumed by individuals were recorded. Snails from both species easily copulated and individuals displayed elaborate stereotyped courtship behaviour. Three distinct phases were observed in the mating behaviour of these two snail species. Mating starts by shell mounting with unilateral intromission, the sexual roles changing after the first mate. In *B. camerunensis* in particular, simultaneous reciprocal mating, characterized by simultaneous intromission of male organs, was observed in few couples. In these two species, a relation between the mating system and the number of copulations was noticed, as a higher number of copulations were recorded in outcrossing (*B. camerunensis*) than in selfing (*B. pfeifferi*) snail species. No preference in the sexual role of mates was observed.

## INTRODUCTION

Planorbidae are one of the most diverse and common worldwidefamilies of Basommatophoran Pulmonates. They are cleverly living organisms able to colonise diverse fresh water habitats and to adapt themselves to environmental changes. These abilities are partly due to their reproductive system which comprises three parts of different ontogeny origin and functions (hermaphroditic, female and male parts). The independent evolution of these parts, which enhance variation in primary sexual characters [1], has provided structural and functional explanations to the observed diversity in mating system and mating behaviour [2,3]. Indeed, their mating behaviour is very rich and diverse, and its taxonomy utility is discussed [4]. Some species mate by shell mounting and display elaborated courtship behaviour [5-8]; others mate face-to-face without any distinct pre-copulatory behaviour [4]. Flat-spire snails can copulate both unilaterally and reciprocally, with simultaneous intromissions.

Data describing mating behaviour of *Biomphalaria* species are very scanty and in Cameroon, no data is even available to our knowledge. *Biomphalaria pfeifferi* and *Biomphalaria camerunensis* are two *Schistosoma mansoni* intermediate hosts found in Cameroon, belonging to the Planorbidae Family. Their mating system (egg production and survival under self- versus cross-fertilization) was recently studied [9]. *B. pfeifferi* appears as a high selfing species [9] and very few copulations were observed in paired snails [10]. At the opposite, *Biomphalaria camerunensis* is a preferential outbred species [9] which exhibits a waiting time when isolated.

These results on mating system have been useful to better understand the epidemiology of intestinal schistosomiasis in Cameroun, and elaborate control strategies against these intermediate hosts. Since mating system is affected by the copulatory behaviour of individuals [11], the aim of the present paper is therefore to describe the copulatory behaviour of these two *Biomphalaria* species so as to highlight the relation between their mating system and copulatory behaviour.

## MATERIALS AND METHODS

### Collection sites

Wild individuals were collected at three different sites: the Afeme River (Nkolbisson, Yaoundé) for *B. pfeifferi*, the Yana Messina stream and the Mounassi swamp (Minkama, Obala) for *B. camerunensis*. They were brought alive to the snail rearing room at the Faculty of Science of the University of Yaoundé I. Their taxonomic status was defined on the basis of shell morphology [12] and of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism analysis (PCR-RFLP) of the ITS2 region [13]. They were maintained at 26 ± 1°C throughout the experiment under a 12L/12D photoperiod. Small floating pieces of polystyrene were introduced in rearing boxes for eggs' laying, and eggs laid were used to develop a new generation (G1). Juvenile of the first generation (G1) were maintained isolated in the rearing boxes until sexual maturity (first-egg-laying). This sexual isolation was made to increase the urge to copulate.

### Behavioural study

Mature virgin young snails of each species were paired together in 5 cm deep containers (2 individuals per box). For photography needs, some observations were done in Petri dishes where the behaviour of the mating snails remains essentially the same as in the larger containers [4]. Mating behaviour was observed in 15 pairs of snails originating from each of the collection site (Nkolbisson for *B. pfeifferi*, Yana Messina and Mounassi for *B. camerunensis*). One individual of each pair was marked using white corrector fluid. This technique is appropriate and reliable since it is known to not consistently affect the life-history traits of marked individuals as previously demonstrated with the freshwater snail *Physa acuta* [14]. Experimental snails were followed up every day from 8 am to 5 pm during three consecutive days, and separated between 5 pm and 8 am of the next day. Snails were not fed during the pairing period. According to the conventional terminology for sexual roles [15], if mating is unilateral, the individual donating the sperm is considered as the 'male', and the individual receiving the sperm is called the 'female'. If mating is reciprocal, the individual that initiates mating and exhibits a more active mating behaviour is referred to as the 'active partner' and the other individual as the 'passive partner. Copulation was considered successful when the male acting individual inserted its preputium into its partner's vaginal pore. Mating process was divided into three consecutive phases (pre-copulation, copulation, post-copulation) as previously described by Trigwell and colleagues [16]. The courtship behaviour, the number of copulations, as well as the sexual role assumed by individuals were recorded.

### Data analyses

All relevant data were recorded into a purpose-built Microsoft Excel database and subsequently exported into PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) for statistical analysis. The frequency of copulation was computed as the percentage of snails copulating among the total number of snails followed, and the sexual expression or sexual role assumed evaluated by taking into account individuals copulating either as male or as female. The 95% confidence interval (CI) for proportions was calculated using the Wilson method not corrected for continuity [17], and Chi-square procedure used to compare these percentages between snail species or populations. The number and duration of copulation were computed as arithmetic means, and the sampling fluctuations estimated using the standard deviation (SD). Mann-Whitney rank test was used to compare these means between snail species and populations.

## RESULTS AND DISCUSSION

Observations of paired individuals during a three-day period indicated that snails from both species have the propensity to copulate as soon as they are put together with a mate partner. It was indeed demonstrated that isolated young snails are eager to copulate as soon as they are paired [5,18]. In both species, individuals displayed elaborate stereotyped courtship behaviour. During the pre-copulatory phase, the male pursued its female partner (Figure 1a), crawled upon its shell and circled several times along the circumference of its upper side (Figure 1b). Then

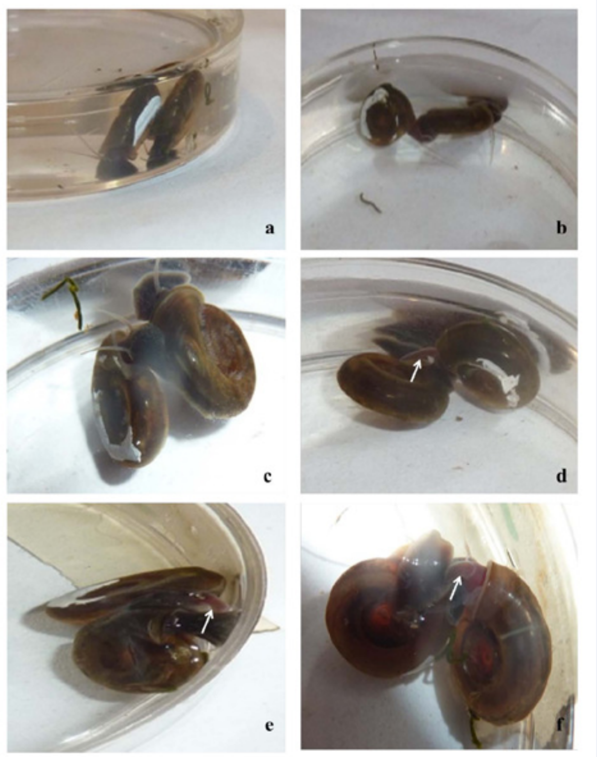


**Figure 1** Mating behaviour sequences in *Biomphalaria camerunensis* and *Biomphalaria pfeifferi.*

a: pursuance and mounting; b: circling; c: positioning; d: eversion of the preputium (the white arrow shows the male preputium searching for the female vaginal pore); e: probing (the white arrow shows the male preputium being inserted in the female vaginal pore); f: intromission (the white arrows indicates the preputium during insemination).

he moved over to the shell's aperture where he affixed itself in such a way that the two partners' heads were orientated towards the same direction as observed in *Planorbis planorbis* [4] (Figure 1c). Tentacles agitation with intersection can be observed in both partners, and there could be a chemical stimulation by the released of secretions from tentacular glands as observed in another Planorbidae *Segmentina oelandica* [19]. The male then slowly everted its preputium (Figure 1d), searching for the female vaginal pore (Figure 1e); when the appropriate position was reached, the intromission occurred (Figure 1f). This intromission process represents the copulation phase. Initially, the everted preputium is white, but during copulation it becomes reddish, indicating the inflow of the haemolymph through the copulatory organ and it readiness for sperm exchange. At the end of insemination, some spasmodic body contractions can be observed, and if the partners are disturbed by mechanical agitation the pair readily breaks apart. During the post-copulatory phase the male individual retracted its preputium, dismounted the female and moved away from her. Many similarities in the mating process were observed in these two biomphalarian species, and can be explained by their closed phylogenetic status [20]. During this mating process, the female acting individual was either attached firmly to the substrate (the wall of the box) with its foot, or the pair floated on the water surface with their shells tightly pressed together. In general, unilateral mating with exchange of the sexual role after the first copulation was observed. Indeed, in each couple, the frequency of individuals copulating with exchange of sexual role after the first copulation (65.8%; 95%CI: 49.9% - 78.8%) was significantly higher than individuals copulating only as male or only as female (34.2%; 95% CI: 21.2% - 50.1%) ($p$ = 0.006). Nevertheless in *B. camerunensis*, another distinct type of copulation, characterized by simultaneous reciprocal mating during which mutual intromissions occurred, was also observed. The two partners were acting both as male and female simultaneously, as was described in *Anisus vortex* [4], *B. glabrata* [21-25], and *Drepanotrema depressissimum* [26]. Differences in the sexual organs [7] and/or in the genetics of *B. camerunensis* can explain this type of copulation [20]. In this case, the two partners were attached by their feet on the wall of the box and their basal sides were facing each other. The active partner evaginated its preputium before the passive ones. When the two penises were evaginated, each partner searched for the female gonopore of the other partner and mutual intromissions occurred simultaneously. The frequency of this phenomenon was rare, representing 1.5% (95% CI: 0.3% - 7.8%) and 10.0% (95% CI: 5.4% - 20.9%) of the total number of copulations reported for Yana Messina and Mounassi snails, respectively.

The number of daily copulations was similar between the two *B. camerunensis* populations (Yana Messina and Mounassi) ($p$ = 0.367), and significantly higher in each of the *B. camerunensis* population as compared to the *B. pfeifferi* population ($p < 0.0001$) (Table 1). This latter result could be due to the difference observed in their mating system, which is preferentially cross-fertilization for *B. camerunensis*, but self-fertilization for *B.pfeifferi*. In fact, the high propensity to copulate characterizes outcrossing species in Planorbidae [16].

No significant difference was found in the sexual expression of individuals, both for *B. camerunensis* ($p$ = 0.377) and *B. pfeifferi* ($p$ = 0.066), as we recorded a similar number of mating either as male or as female. This can be explained either by an optimal allocation between male and female functions [1] or by our experimental design. The fact that these species are true simultaneous hermaphrodites, as compared to protandrous Planorbidae species, can explain why rejection behaviour was not observed. In simultaneous hermaphrodites, there are more complex gender relations (sexual conflicts) than in protandrous hermaphrodites, especially in the snails with exclusively unilateral copulation [4].

The mating duration was quite long and similar between these two species ($p$ = 0.227), lasting 30 min to 2 hours (mean: 1.27; SD: 0.30), and 1 to 5 hours (mean: 2.33; SD: 1.70) for *B. pfeifferi* and *B. camerunensis*, respectively. The shorter duration of mating in *B. pfeifferi* can be explained by its mating system, since copulation in self-fertile species does not really contribute to the reproductive success of individuals. In general, a longer duration of mating was recorded in the present study, as compared to that observed by Sodatenko and Petrov [4] in six other Planorbidae species, and can be explained by the phylogenetical distances between these species [20].

## CONCLUSION

This study has shown that copulations occur both in highly selfing (*B. pfeifferi*) and highly outcrossing (*B. camerunensis*) species, although with higher rate in *B. camerunensis* as compared to *B. pfeifferi*. The most interesting observation was the simultaneous mating through mutual insemination in few *B. camerunensis* couples. An anatomical description of the reproductive organs of this species may be useful to fully understand this type of mating. The higher number of copulations and the mating behaviour of *B. Camerunensis* are in favour of a high genetic diversity, thus constituting an asset in reducing the susceptibility of this snail species to *S. mansoni*. Therefore it could be of interest, while defining control measures against schistosomiasis, to determine for instance genes linked to resistance in *B.camerunensis* populations and introduce them in susceptible *B. pfeifferi* populations.

**Table 1:** Total number of copulation according to snail species and populations.

| Snail species | Snail population | No. couples | Mean | SD | 95% CI for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound | | |
| *B. camerunensis* | Yana Messina | 15 | 4.60 | 1.29 | 3.88 | 5.32 | 2 | 7 |
| *B. camerunensis* | Mounassi | 15 | 4.27 | 1.22 | 3.59 | 4.94 | 2 | 7 |
| *B. pfeifferi* | Nkolbisson | 15 | 1.53 | 1.19 | 0.88 | 2.19 | 0 | 4 |

No.: number of; SD: standard deviation; 95% CI: 95% confidence interval; Min: minimum; Max: m

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