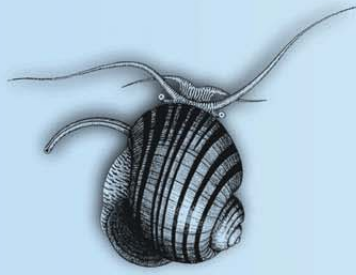




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Penis-rejection in a mangrove littorinid snail: why do females reject males?

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Females are generally more choosy than males in their choice of mates but the underlying causes for this behavioural difference between the two sexes are yet to be resolved (Schärer, Rowe & Arnqvist, 2012; Ah-King, 2013; Kokko, Booksmythe & Jennions, 2013). In cases where female mate choice occurs, this behaviour is often interpreted as a mating preference whereby females increase their fitness by receiving direct (e.g. improved territory quality or paternal care) or indirect (e.g. good genes) benefits from mating with 'high quality' males (Andersson, 1994). Female mate choice may also, however, evolve through sexual conflict if mating is associated with fitness costs for females (e.g. increased risk of predation, decreased foraging time or physical damage). Female fitness may, for example, decline when the costs of mating exceed any benefits accrued from receiving additional sperm (i.e. excessive matings) (Arnqvist & Rowe, 1995). In such cases, females may evolve some means to limit mating to avoid sexual harassment or an excessive number of mating events (Arnqvist & Rowe, 1995).

Although most marine snails are dioecious and have internal fertilization (Baur, 1998), female mate choice has rarely been recorded in these animals (but see Miranda, Lombardo & Goshima, 2008; Lombardo & Goshima, 2011). In contrast, male mate choice has been recorded in many littorinid snails (Saur, 1990; Erlandsson & Johannesson, 1994; Ng *et al.*, 2013; Saltin, Schade & Johannesson, 2013) and in the Neptune whelk, *Neptunea arthritica* (Lombardo & Goshima, 2011; Lombardo *et al.*, 2012). Similar to insects, many marine snails are promiscuous and females are able to store sperm after copulation (Baur, 1998) and hence there is also the potential for cryptic female choice (i.e. differential sperm usage by females); however, as yet there is no empirical evidence for this process (Panova *et al.*, 2010).

Littoraria melanostoma (Gray, 1839) is an intertidal snail that is found in low densities in the canopies of mangrove trees in Hong Kong. These littorinids have a narrow window of activity, which is mainly associated with cooler temperatures at night and moisture brought by the tide and rainfall (Lee & Williams, 2002a, b). Females of *L. melanostoma* exhibit a penis-rejection behaviour, in which the female bends her head and extrudes her snout to push the penis of males attempting to copulate with her away from her mantle cavity (see video in Supplementary material), which we hypothesized could be associated with female mate choice. As mating in littorinids involves the male mounting the female's shell, and *L. melanostoma* females often slow down or stop crawling during this process (T.P.T. Ng, personal observation),

mating might, therefore, invoke a fitness cost to the females by decreasing their foraging time. Mating may also increase the risk of detachment from the substratum and hence increase the risk of crab predation (Johannesson *et al.*, 2010). Scars on the shells, which appear to be the result of unsuccessful crab attacks, are commonly found in *L. melanostoma* and indicate the risk of predation in this species (T.P.T. Ng, unpubl.). Therefore, we first tested the hypothesis that females might reject males that could increase the risk of detachment or reduce the females' foraging time, based on the males' body size (e.g. females may reject large, heavy males or males larger and heavier than themselves). We also tested a second hypothesis that, if mating is not excessive (i.e. females have not experienced any mating attempts prior to the mating season), females would be unlikely to reject copulation attempts by males.

To test the first hypothesis, a laboratory experiment was conducted to investigate if females would reject large males or males larger than themselves (i.e. they were choosy about the size of male they would allow to copulate with them). Mature snails (>11 mm, Lee & Williams, 2002a) were collected from Tsim Bei Tsui mangrove, Hong Kong (22°29'N, 114°00' E) in May to June 2012 and divided into two size categories (small: 14–18 mm; large: 23–27 mm). A small or a large male was placed with either a large or a small female in a spherical container (diameter: 50 mm) with a mist of Milli-Q water (sprayed hourly) in each trial (see Ng & Williams, *in press*). Under such conditions, males generally initiated copulation with females within an hour and, if no copulation attempt occurred within 1.5 h, the trial was aborted. Copulation duration (measured from when the penis entered the mantle cavity of the female until the tip of the penis left the mantle cavity; Saur, 1990) and any female penis-rejection behaviour were recorded in 22 replicates of each of the four possible female-male size combinations (large female–large male, small female–large male, large female–small male and small female–small male, $\sum n = 4 \times 22 = 88$).

Penis-rejection behaviour was found in most cases (>85%) in all the four size combinations (Table 1) and so measurement of copulation duration was only possible in a few cases. In the eight cases without penis rejection, copulation durations were highly variable, ranging from 54.3 to 210.8 min (Table 1) and males left females immediately after withdrawing their penis. We therefore also recorded the duration for which males stayed in the copulation position in the cases where penis rejection was recorded, which did not vary among the four size combinations (one-way ANOVA: $F_{3,76} = 0.466$, $P > 0.05$; Table 1). After

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Table 1. Occurrence of penis-rejection behaviour, duration that males stayed in the copulation position with females (only the cases in which penis rejection occurred) and copulation durations of the four female-male size combinations of *Littoraria melanostoma*.

Size combination	Occurrence of penis rejection	Mean duration males stayed in copulation position (\pm 95% confidence interval, min)	Maximum duration males stayed in copulation position (min)	Copulation durations for the cases without penis rejection (min)
LF-LM	20 (90.9%)	139.7 \pm 58.2	480.4	83.3, 131.2
SF-LM	21 (95.5%)	126.0 \pm 48.8	358.2	79.7
LF-SM	20 (90.9%)	168.1 \pm 64.9	420.8	72.4, 210.8
SF-SM	19 (86.4%)	150.9 \pm 48.5	341.4	54.3, 56.0, 159.4

Abbreviations for size combinations: LF–LM, large female–large male; SF–LM, small female–large male; LF–SM, large female–small male; SF–SM, small female–small male.

initial rejection, all males stayed in the copulation position and kept attempting to reinsert their penis, but females continued to reject reinsertion of the penis (see video in Supplementary material).

To test the second hypothesis, mature female *L. melanostoma* (18–22 mm) were tagged and placed on two isolated mangrove trees from which all males had been removed three months before the start of the mating season in April (Ng & Williams, 2012), so it could be reasonably assumed that these females had not experienced excessive matings. Females were then collected and returned to the laboratory where they were allowed to mate with males of a similar size (18–22 mm) using the methods described above. In the majority of cases (90%, 18 out of 20 trials) these females still showed penis rejection behaviour.

There was, therefore, little support for the hypotheses that penis rejection behaviour in *L. melanostoma* was associated with female mating preference for males of a certain size, or to avoid excessive matings. Penis rejection also did not reduce the time that male *L. melanostoma* stayed with females, as the time males were mounted on the females did not vary between mating pairs with or without penis rejection (Table 1).

The gradual reduction in the frequency of penis insertion by males, and in the head bending with snout extrusions by females, in all trials suggest that the continuous rejection and insertion of the penis is energetically costly for both males and females (T.P.T. Ng, personal observation). If the purpose of penis rejection is to reduce any costs due to being mounted by a male (e.g. reduced foraging time or increased risk of detachment from the tree), we would expect a more effective mechanism to avoid males or ‘convenience polyandry’ (i.e. females accept the costs of excessive matings instead of spending time and energy rejecting the males that ‘harass’ them; Thornhill & Alcock, 1983). Convenience polyandry has been suggested for another littorinid, *Littorina saxatilis* (Panova *et al.*, 2010), which is found in high densities on rocky shores. In *L. saxatilis* mating has been suggested to incur costs for the female (increased risk of dislodgement by waves) and females may, therefore, suffer from excessive and costly matings in this abundant species (Johannesson *et al.*, 2010). Female *L. saxatilis* avoid excessive matings by masking their identity in their mucus trails, reducing the chances of males locating them via trail following. Females, however, did not reject any of the males that located and initiated copulation with them (Johannesson *et al.*, 2010). In contrast, *L. melanostoma* occurs at low densities in complex mangrove-tree habitats (Lee & Williams 2002b), where it may be assumed that mate encounter rates are low, suggesting that females of this species are unlikely to suffer from excessive matings. Females of *L. melanostoma* also do not mask their identities in their mucus trails (i.e. males are able to discriminate and follow female trails; Ng *et al.*, 2011), which further supports the theory that penis rejection is not associated with avoidance of excessive matings in this species.

Frequent penis rejection (i.e. over 65% of copulations) has also been reported in *N. arthritica* (Miranda *et al.*, 2008). In this

species female behaviour was more aggressive, the female biting the penis and foot of the males, and most males quickly aborted copulation attempts (Miranda *et al.*, 2008). Some male *N. arthritica* were, however, able to tolerate the females’ rejection attempts and copulated with the females for long durations (Miranda *et al.*, 2008; Lombardo & Goshima, 2010). Miranda *et al.* (2008) therefore suggested that the attempted rejection might serve the purpose of ‘testing’ the quality of potential mates. Radula rasping of the male’s penis was also observed during the rejection behaviour in *L. melanostoma*, but no obvious bite marks or wounds were visible. Given that female *L. melanostoma* did not seem to wound the males’ penis, and that the process of rejection and insertion of the penis seemed to be energetically costly, this behaviour may well be a strategy for females to select ‘good-quality’ males. In fact, males that attempted copulation for longer durations in the experimental trials seemed to have a higher chance of fertilizing females, as females often did not reject the males’ penis immediately after the males reinserted their penis, and they often stopped rejection after an extended period (T.P.T. Ng, personal observation). Further investigations are, however, needed to investigate fertilization success under intensive rejection attempts and whether certain males are more successful than others in fertilizing females. The role of penis-rejection behaviour in *L. melanostoma*, therefore, deserves further exploration, as it could provide new insights into the importance of sexual selection in marine snails, especially in terms of female mate choice, which has been largely ignored.

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