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Mucus trail following as a mate-searching strategy in mangrove littorinid snails

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Mate searching often involves chemical cues and is a key process in determining fitness in most sexually reproducing animals. Effective mate-searching strategies are, therefore, essential for individuals to avoid wasting resources as a result of misrecognition of mating partners. Marine snails in the genus *Littoraria* are among the most successful molluscan groups that live closely associated with mangroves. Their population densities are often low, and finding a mate within the complex three-dimensional habitat of tree leaves, branches and trunks requires an effective searching strategy. We tested whether males of *L. ardouiniana* and *L. melanostoma* located females by following their mucus trails. In the laboratory, male tracker snails followed mucus trails laid by conspecific female marker snails at a higher intensity compared with other marker–tracker sex combinations in the mating season, but not in the nonmating season, and this was more pronounced in *L. ardouiniana*. Male trackers did not move faster when following the trails of conspecific female markers compared with other sex combinations; however, tracker snails moved faster in the mating than in the nonmating season, although this might be related to temperature. In both species, males tracked females regardless of trail complexity, and the majority of male trackers were able to detect the direction (polarity) of the trails of conspecific females. Together with previous studies on rocky shore *Littorina* species, these findings suggest that sex pheromones are incorporated into mucus trails to facilitate the reproductive success of these snails. Mucus trail following is, therefore, an adaptive mate-searching strategy in intertidal gastropod molluscs, and potentially in other gastropod groups in which trail-following behaviour is prevalent.

Along with foraging or predator avoidance, finding a mate is a key behaviour that determines the fitness of an individual (Krebs & Davies 1993). In most internally fertilizing dioecious species, reproductive success is strongly determined by the ability to search for mates of the right species and sex (Parker 1978). Selection for reproductive traits that will facilitate successful mate location is, therefore, likely to reduce the time and effort wasted in locating and 'copulating' with organisms of the wrong species and sex (Andersson 1994). mate-searching mechanisms vary among species, but are generally energetically costly activities that involve physical movement and other forms of behaviour such as the production of chemical cues (Kokko & Wong 2007). Chemical cues involved in mate searching (sex pheromones) have been extensively studied and reported across animal taxa, especially in insects (Mayer & McLaughlin 1990; Landolt 1997), and are the subject of increasing attention in other animal groups such as molluscs (Susswein & Nagle 2004). Apart from releasing pheromones directly into air or water, animals often incorporate their pheromones into excretory fluids such as urine, sweat and mucus (Law & Regnier 1971; Wyatt 2003; Tirindelli et al. 2009). The study of how animals utilize different media to transmit their sex pheromones is, therefore, fundamental to increasing our understanding of sexual communication in animals (Cardé & Baker 1984; Tirindelli et al. 2009).

Gastropod molluscs are important components of intertidal communities (Underwood 1979). Mate searching for these animals can be difficult, however, as intertidal habitats often support substantial numbers of co-occurring species, the activity windows of which are usually synchronized to specific tidal conditions (Little et al. 2009). Snails in the family Littorinidae are among the most common intertidal molluscan groups. They are widely distributed throughout tropical and temperate regions, and play a significant role in the ecology of intertidal communities (Reid 1989; McQuaid 1996). Their abundance and ubiquitous distribution make these snails excellent models to study mate-searching strategies in intertidal gastropod molluscs.

It has been suggested that members of the rocky shore genus *Littorina* incorporate pheromones in their mucus trails to facilitate mate searching (Struhsaker 1966; Dinter 1974; Saur 1990;

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Erlandsson & Kostylev 1995; Johannesson et al. 2010). Johannesson et al. (2010), for example, demonstrated that during the mating season, males of three *Littorina* species (*L. littorea*, *L. fabalis* and *L. obtusata*) followed mucus trails laid by conspecific females for longer distances than mucus trails laid by conspecific males. Males of another rocky shore species, *Littorina saxatilis*, were even able to discriminate between mucus trails laid by conspecific females of different ecotypes (Johannesson et al. 2008). Despite this extensive work on the rocky shore genus *Littorina*, it is unknown whether mucus trail following is an important strategy for mate searching in other members of the family Littorinidae, particularly those from different habitats.

Within the Littorinidae, snails in the genus *Littoraria* are among the most successful gastropods in mangrove forests in the Indo-Pacific region (Reid 1986; Reid et al. 2010), where they play an important role in food web dynamics (Alfaro 2007, 2008). *Littoraria* often mate in a defined season, and males actively search and mount females (Gallagher & Reid 1974). Population densities of these snails are often low in mangroves, however (Reid 1985; Lee & Williams 2002b; Sanpanich et al. 2008; Torres et al. 2008), and searching for mates in the complex three-dimensional habitat of tree leaves, branches and trunks would appear to be extremely difficult without any specific mate locating strategies.

In this study, we investigated mating behaviour in the most abundant littorinids found in the tree canopy of Hong Kong mangroves: *Littoraria ardouiniana* (Heude 1885) and *Littoraria melanostoma* (Gray 1839) (Lee & Williams 2002b). Males of these two species search for, and mate with, females most intensively during the hot, wet summer, and the frequency of males forming pairs with males or heterospecific females is very low (Ng & Williams, unpublished data). On this basis, it was hypothesized that trail following could be an effective mate-searching strategy adopted by males to identify and locate conspecific females. To test this, experiments were conducted in the laboratory to investigate various aspects of mucus trail-following behaviour in relation to the mating and nonmating seasons, to determine the role of mucus trail following in mate searching in these mangrove snails.

METHODS

Animal Collection

Large (21–26 mm) mature (>12 mm; Lee & Williams 2002a) *L. ardouiniana* and *L. melanostoma* were collected from *Kandelia obovata* trees at Tsim Bei Tsui (22°29′N, 114°00′E), in outer Deep Bay, NW New Territories, Hong Kong. Snails were sexed in situ by turning their shells to determine whether the snails had a penis, and sexes were held separately to prevent mating.

In the laboratory, male and female snails were kept in separate tanks at 27–29 °C in the mating season (July to August 2009, during Hong Kong's hot and wet season; see Kaehler & Williams 1996) and at 18–20 °C in the nonmating season (December to January 2009, during Hong Kong's cool and dry season) without water and food overnight. All experiments were conducted on the day after collection. Similar to other littorinids (e.g. *Littorina subrotundata*; Zahradnik et al. 2008), males of *L. ardouiniana* and *L. melanostoma* frequently mate with females on consecutive days after collection from the field (T. P. T. Ng, unpublished observations). Mating before experiments was, therefore, not assumed to be a significant confounding factor for the mucus trail-following behaviour of the snails.

Trail Following

Various combinations of the same or different species, or sex, of snails were tested to determine responses to different mucus trails. Experimental conditions were based on the environmental conditions animals experienced in the field, where activity is mainly elicited by rain and moisture brought by the tide and dew, and where submersion by sea water is generally avoided (Reid 1985; Yipp 1985; Lee & Williams 2002a). To achieve this, snails were activated by spraying with double distilled water (Little & Stirling 1984). Light was primarily provided by overhead fluorescent lights and laboratory windows were covered with blinds to minimize external stimuli. Experiments were conducted in the same room, and therefore at the same temperature, as the snails were stored.

Three temporal replicate series of experiments were conducted for both *L. ardouiniana* and *L. melanostoma* in both the mating and nonmating season. In each set of experiments, snails were randomly assigned as markers and trackers. Markers were snails used to crawl and lay a mucus trail, whereas trackers were the snails that were placed onto the starting point of the marker's mucus trail to allow trail following. In total five marker–tracker combinations were tested for each species, namely Male–Male; Female–Male; Male–Female; Female–Female and Heterospecific Female–Male, with 10 replicate trials for each combination ($\Sigma N = \text{season} \times \text{sex combination} \times \text{replicate series} \times \text{replicate} = 2 \times 5 \times 3 \times 10 = 300$).

Experiments were conducted on a plastic board (60 × 60 cm) with a test arena of 50 × 50 cm and a fixed, marked centre point. In each trial, a clean acetate sheet was attached to the plastic board and a mist of double distilled water sprayed onto it. The marker snail was placed at the centre and allowed to crawl until it reached the boundary, where it was removed. Using this method, the snail's trail was clearly visible. The acetate sheet was then sprayed with double distilled water to homogenize the surface and the tracker snail was then introduced. To avoid errors caused by body orientation after attachment, the tracker snail was allowed to attach onto a small moist acetate disc and then positioned so that it would crawl onto the starting point of the marker trail. The tracker was allowed to crawl until it reached the boundary of the arena, where it was removed. During the experiment, the trail-following time (time moving on the marker snails' trail) of the tracker snails was recorded. The marker snail's distance and the overlapping distance of the marker and the tracker trails were traced using a digital map measurer (Yorter Model No. V-930, accuracy: ±1 mm). The net displacement (direct line from the initial point to where the snail left the arena) of the marker trail was measured using a ruler (±1 mm). The experimental order for each replicate trial was randomized and each snail was used only once, with clean acetate sheets for each experimental trial. The tortuosity index (TI; net displacement/distance travelled) of the marker and the coincidence index (CI, distance followed by tracker/total trail distance of the marker) were scored following Davies & Beckwith (1999). $TI = 1$ when the marker produces a straight trail, and the more complex the trail becomes, the smaller the value of TI. Trackers that completely follow a marker score a CI value of 1, and 0 when they do not follow the marker at all.

Polarity

To determine whether males can detect and respond to the direction (polarity) of conspecific females from their mucus trails, another trail-following experiment was conducted during the mating season. An acetate sheet was evenly sprayed with a mist of double distilled water and a female marker snail was placed at one end of the sheet and allowed to crawl towards the opposite end. The marker snail was then removed and the acetate sheet was randomly rotated in either direction through 90° and sprayed with double distilled water to homogenize the surface. A male tracker