Timing the start of fertilisation in *Littorina saxatilis*

BACKGROUND

It is not uncommon for closely related animals and plants to have diverged in the way that sperm and pollen are transferred. This form of reproductive isolation is known as gametic or gametophytic isolation (Dobzhansky 1951) and it represents one barrier that can be responsible for the nonrandom union of gametes from two parental populations (Markow 1997). For example, it has been shown between different species of *Drosophila* that heterospecific sperms were less motile and stored in smaller quantity in the female organs than the conspefic sperms (Patterson 1947). Gametic islation can also occur between distinct populations of the same species and potentially contribute to the formation of new species. Self-incompatibilty is one major mechanism acknoledged in plants and there are a few studies that have focused on inbreeding in animals (Markow 1997; Ober et al. 1992) and fewer that have studied the consequences of a fertilisation barrier to the likelihood of speciation (for an example of such a study, see Larson et al. 2011).

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Fertilisation involves mutliple steps due to the complex interactions between male and female post-copulatory reproductive traits (reviewed in Wolfner 2009).

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Females and males of the intertidal snail species *Littorina saxatilis* adopt a characteristic mating position that can be clearly observed in the wild as well as in the lab. Typically, the male approaches the female and crawls on top of her shell until he stops at the front-right side of the female shell. At this specific mounting position, the male can insert the penis under the female shell and initiate transferring the sperms. When the penis is inserted is difficult to establish but it has been found a strong correspondece between male mounting position and copulation attempt (Hollander et al. 2005). What has not been investigated in *L. saxatilis* is the starting point at which the sperms are being transferred into the female reproducitve tract.

There are two main reasons why we examined the time of sperm transfer in the *Littorina saxatilis* system. The first one is that the rough periwinkle is a brooder and the females can carry up to ... (ref. for number of offspring) that at the end of their embryonic development (ref. for development time) crawl away from the mother as juveniles. This means that we can infer whether the sperms have been transferred by identifying the stage of the eggs after dissection of the female storage organ. The second reason is connected with the results by Panova and colleagues (2010). They found an extreme level of promiscuity and a deviation from the expected number of offspring per sire. However, they were uncertain whether it was a result of postcopulatory sexual selection (e.g., cryptic female choice and/or sperm competion), a process often involved during the build-up of reproductive isolation. To test for this requires the knowledge about how and when the sperms start to move into the female reproducitve tract. Here, we focus on the when, essentially the time at which the fertilisation begins in *L. saxatilis*. Under laboratory conditions, mating pairs of the marine snail species *L. saxatilis* have been observed to mate for different durations. Interestingly, the relationship between number of matings and duration contains two peaks, one at approximately five minutes and one at around 30 minutes after the male had assumed the characteristic mounting position (Fig. 1). We aborted copulations at one, five and 30 minutes in order to understand fertilisation and offspring production in *L. saxatilis*. Short copulations (one minute) are expected to be inadequate for sperm transfer whereas longer copulations (30 minutes) are more likely to yield over time an effective transfer of sperms. Finally, intermediate copulations (five minutes) will be relevant for the description of postmating prezygotic patterns. These may show whether females will produce offspring and if so whether they will produce fewer than the females mated for 30 minutes.

Figure 1. Count of embryos in the control and three different mating time groups of a pilot study.

HYPOTHESIS

Females of other littorinid species can receive a quantity of sperms that is proportianal to the mounting duration (Hollander et al. 2018). An equivalent relationship is likely to apply to *L. saxatilis* and we expect

that very short matings (one minute long) will not be sufficient for the sperms to travel through the female reproductive organs and eventually fertilise the eggs.

QUESTION
When does sperm transfer start in *L. saxatilis*?

MATERIALS AND METHODS (for now, look at the schedule file)
Wild males were sampled from a rocky shore on the island of Saltö which hosts regularly a suitable variation of shell size. Females were instead reared under laboratory conditions isolated from males as unobserved matings will confound the level of paternity. Females and males were sized and then each female was matched once with a male according to the estimated optimal size ratio (ref.). If no copulation attempt had been observed throughout the length of the experiment (TBD), the same female was used the next day and paired with another male under the same method.
Virgin Crab and Wave ecotype females will be placed individually in plastic spheres (diameter) and let them mate with slightly smaller males for one, five or 30 minutes. Aborted copulations will be performed for all the three groups at the respective times. Reproductive events that will last less than the pre-assigned time will be also recoded and females included in dissections. Dissections of the females will be planned two-three weeks after the breeding session of the experiment when embryogenesis will have reached suitable stages for the discrimination between uncleaved eggs and developing embryos.

References

Dobzhansky, Theodosius. 1951. *Genetics and the Origin of Species*. 3rd ed. Vol. 11. Columbia University Press.