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Rearing System and Behavioural Observations for *Lumbricus terrestris*

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Introduction

The earthworm *Lumbricus terrestris* serves as a model for the study of mating systems in outcrossing simultaneous hermaphrodites. It has a long lifespan of several years and a slow reproductive rate with high investment in single offspring. Being an anecic species, it lives in single vertical, seldomly Y-shaped burrows. Copulations take place on the soil surface and pose a serious predation risk. *L. terrestris* is parasitized by the gregarine protozoan *Monocystis sp*. which lives mainly in the seminal vesicle where self-sperm is stored. It usually does not harm its host, but castrates the worm when parasite cysts occur in high densities. All reproductive features of *L. terrestris* lead to the hypothesis that they should exhibit mate choice. In contrast to gonochoric species, mate choice scenarios can look different in hermaphrodites as they unite male and female interests in one body during a single copulation. Little is known about interactions between several *L. terrestris* living in a group, as is the case in their natural habitat. We study these interactions in a laboratory experiment using video observation.

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Materials and Methods

Earthworms were kept in artificial burrows made of cable ducts. These can be opened to check the worms on a regular basis. They are filled with loamy soil that was dried, rewetted and passed through a mesh. This rather liquid substrate is then mixed with coconut fibre. The latter prevents moisture loss and fungal growth. Filled cable ducts are closed at one end with duct tape to prevent escapes. To simulate ground water levels, the duct tape is punctured and this end of the cable duct is placed into dH_2O. The worm is inserted into the soil from the top, allowing it to dig its own burrow. The cable ducts can be placed into different treatment groups: single, in pairs or in groups of six.

Adult *L. terrestris* were purchased from a fish bait supplier and kept singly for 6 weeks.

Before the start of the experiment, they were weighed and their cocoon production was assessed by sieving through the substrate in their burrow. They were then randomised by weight and transferred to new burrows, which were arranged in groups of six worms in a hexagon. A total number of 126 *L terrestris* were used to create 21 replicates. Earthworms are kept in a climate chamber at 15°C and 80% humidity and sprayed daily with dH_20. They are fed regularly twice a week with a handful of frozen lettuce per hexagon. Behaviour is recorded using time lapse infrared recording from 6:30 pm till 5:30 and videos are checked for worm escapes or relocation into other burrows every day to prevent losing track of earthworm IDs.

Results

We observed food stealing between burrows, but also novel aspects of their mating behaviour. The hexagonal set-up provided more space for a mating pair than the usual pair set-up. We could observe that copulations do not necessarily take place directly between the burrows, but also in other angles. The most surprising behaviour was the persistent interference by other worms in ongoing copulations (Fig.1). A preliminary data analysis showed that in 69% of the copulations, at least one other worm scanned along the mating pair. In two extreme cases, all four other worms interacted with the mating pair. In 49% of all copulations, the mating pair directly terminated their copulation after contact with a third worm.

Fig. 1 Interaction of 2 worms with a copulating pair

Conclusions

The artificial burrow system is suitable for the observation of *L.terrestris* surface behaviour. It could also be advantageous for ecotoxicological studies as one could add reproductive behaviour as a response. It also reduces the amount of substrate that is needed for keeping the worms and makes the search for cocoons easier. This ongoing study will show whether interference of other worms has an effect on mating duration.

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