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Nest and Feeding Chamber Construction for Cocoon Incubation in the Tropical Earthworm: *Pontoscolex corethrurus*

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ABSTRACT

Details on how earthworm cocoons are deposited in soil have not been documented, nor whether they are abandoned or protected. This study provides the first detailed description of nest- and feeding-chamber construction for tropical earthworm species. The reproductive behaviour of the earthworm *Pontoscolex corethrurus* was observed using 11 "Evans Box" terraria, including one earthworm each. During the progression of the reproductive behaviour of *P. corethrurus*, observations were made at regular intervals for a period of 120 days. *P. corethrurus* built nest chambers with the mouths, coating them internally with a compact layer of fine soil and mucus. One cocoon was deposited in each nest chamber, then suspended from the nest walls by mucus filaments. During incubation, *P. corethrurus* frequently entered nest chambers for repairing or cleaning the inner walls. Besides, earthworms left no casts on the soil surface, but deposited them into underground nests built close to the site where the cocoon was deposited, and entered these underground nests to feed. Juveniles that emerged left the nest to feed on casts deposited in the feeding chamber. Our observations suggest that *P. corethrurus* displays parental care by building and caring for nest and feeding chambers, an aspect which deserves further investigation.

Keywords: behaviour, cast, mucus, Oligochaeta, reproduction, terraria

INTRODUCTION

Earthworms belong to the class Oligochaeta (Annelida: Clitellata); in most soil types they dominate the soil fauna and have inhabited terrestrial environments for over 600 million years (Stephenson 1930; Lavelle and Spain 2001; Coleman *et al*. 2004). They have been referred to as "ecosystem's engineers" for their abundance and biological activity in soil (Jones *et al*. 1994; Lavelle and Spain 2001; Hastings *et al*. 2007).

Most earthworm species display sexual reproduction, although some are parthenogenetic; mating takes place mostly in soil throughout the year, except under unfavourable environmental conditions; some species, such as *Lumbricus terrestris*, mate on the soil surface; the cocoon contains a nutritive albuminous fluid produced by clitellar gland cells located in the anterior part of the body (only in mature worms), and both ova and spermatozoa are discharged into it as the tube passes the spermathecal openings. Fertilization is external, taking place inside the cocoon. The earthworm sheds the cocoon by sliding it over the peristome (Stephenson 1930; Lee 1985; Edwards and Bohlen 1996; Lavelle and Spain 2001; Coleman *et al*. 2004). However, details on how cocoons are deposited in soil have not been documented, nor whether they are abandoned or protected; the only aspect reported is that cocoons possess an extraordinary capacity to withstand drought (Holmstrup and Zachariassen 1996) and infections, and that cocoon production and incubation duration vary with species, soil temperature and moisture (Stephenson 1930; Lee 1985; Edwards and Bohlen 1996). To date, no reports are available on whether earthworms have any behaviour patterns to help increase offspring survival (Ortiz-Ceballos and Fragoso 2006; Grigoropoulou *et al*. 2008).

Pontoscolex corethrurus Muller, 1856 (Glossoscolecidae) is a primarily parthenogenetic earthworm species commonly found in disturbed and natural tropical ecosystems, and is widely tolerant to edaphic and climatic changes (Lavelle *et al*. 1987). *P. corethrurus* is an endogeic species that ingests large amounts of soil with a preference for rich organic soils, continuously building mostly horizontal branching burrows (Lavelle *et al*. 1987). Ortiz-Ceballos *et al*. (2005) and Ortiz-Ceballos and Fragoso (2006) found cocoons of *P. corethrurus* and *B. pearsei* inside well-defined nest chambers, and these cocoons rapidly dehydrated when kept in the open air.

The aim of the present study was to provide a detailed description of nest chamber construction by *P. corethrurus*, to address the question of how earthworms build the nests where cocoons are deposited. Our hypothesis is that nest chambers are built with earthworm cast.

MATERIALS AND METHODS

P. corethrurus earthworms were collected by manual hand sorting from a mango plantation located 79 km southwest (17° 48′ N, 93° 28′ W) of Villahermosa, Tabasco, Mexico. Earthworms were reproduced inside a box containing soil from the collection site, at 25 to 27°C. Earthworms used for this study were obtained from this box. The reproductive behaviour of *P. corethrurus* was investigated in earthworms transferred to eleven terraria (52 × 36 cm) made of two flat glass sheets separated by 5 mm-thick polystyrene pieces placed around the edges (Evans 1947; Capowiez 2000). One kilogram, equivalent oven-dry weight, of well-mixed soil (26.9% clay, 31.6% silt, 41.5% sand and 2.7% organic matter) was placed in each terrarium and moistened. One sub-adult (without a developed clitellum) of *P. corethrurus* was placed in each terrarium. To each side of the terrarium a clear acetate sheet was placed to draw the location of cocoons and label each one with a number by order of appearance. The dates in which each cocoon appeared and juveniles hatched were registered. Nest-chamber and cocoon diameters were measured with a digital calliper. Hatched juvenile behaviour was observed throughout the first 15 days of life.

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The reproductive behaviour of *P. corethrurus* was observed for a period of 17 weeks, until 50% of cocoons in each terrarium hatched. During the first 24 h, observations were made at 30-min intervals, for a least 10 min. Then, at 1-to-6 hour intervals for the next six days; finally, during the remaining 16 weeks the behaviour was observed each Monday from 9:00-11:00 and 13:00-15:00.

Terraria were placed in an incubator at 25 ± 1°C and moistened with water every 5-7 days to maintain field moisture capacity (42%). No food was added.

After 120 days all terraria were destructively sampled. The glass sheets of the terraria were separated to collect earthworms, cocoons and casts. Hatched juveniles and cocoons were collected from soil by hand sorting, counted and weighed. The cast was collected and separated with tweezers. Surface cast, inner cast surrounding nest chambers (within a distance of 2 cm from the nest) and inner cast far from incubation (> 2 cm away from the nest) were dried (63°C) and weighed.

Some important events, such as the construction of nest chambers and the deposition of a cocoon that occurred during the observation of *P. corethrurus'* reproductive activities were recorded using a video camera set in the night-shot mode. The observed behaviour was described based on the data so obtained of 11 earthworms in terraria.

RESULTS AND DISCUSSION

All earthworms placed in each of the terraria survived and grew during the 119 days of experimental manipulation. Mean individual fresh weight increased from 0.214 ± 0.092 g at baseline to 0.409 ± 0.105 g at the end of the experiment.

During the first 7 days, *P. corethrurus* consumed soil, built burrows and deposited cast on the soil surface. The mean (± SD) weight of dried cast found on the soil surface was of 12.45 ± 5.41 g (n = 11) per terrarium. The low production of cast could result from low bulk-density soil in the terrarium, so earthworms could force their way through it without ingesting soil (Topoliantz and Ponge 2005).

We observed that *P. corethrurus* deposited a coating of fine soil particles and mucus in the burrows with the mouth. Burrow walls displayed little porosity, seemingly as a result of being coated with cast and compacted by the radial pressure exerted by both the earthworm's body and constant use (Görres *et al*. 1997; Keudel and Schrader 1999; Görres *et al*. 2001; Jégou *et al*. 2001; Bastardie *et al*. 2005). Afterwards, *P. corethrurus* moved along burrows, likely for repairing (by depositing fine soil particles) or cleaning (by feeding on microorganisms). Schrader *et al*. (2007) reported that burrow walls have a homogeneous and dense arrangement of silt particles which reduces the movement of solutes, water and gases. Also, it is known that earthworms feed on fungi, bacteria, nematodes and collembola growing inside the burrows they build (Devliegher and Verstraete 1997; Brown *et al*. 2000; Brown and Doube 2004).

During a period ranging from 8 to 48 days, *P. corethrurus* consumed soil, leaving irregular spaces where casts were deposited inside a new nest chamber, and not on the soil surface or inside burrows, as suggested by Jégou *et al*. (2001, 2002). Afterwards, they returned frequently to these chambers to feed soil from casts and deposited fine soil on the burrow's inner walls. This explains the suggestion of Jégou *et al*. (2001) and Schrader *et al*. (2007) that cast are used for coating the burrow inner walls. This behaviour suggests that, during the reproductive phase, *P. corethrurus* may build feeding chambers to facilitate the development and growth of microorganisms and nutrients from the breakdown of organic matter in cast. The average weight of inner-burrow cast away from nest chambers was 42.13 ± 19.62 g (n = 11) per terrarium.

In days 49 to 57, *P. corethrurus* started to deposit the first cocoon; during the following 20 days, they produced an average of 4.0 ± 1.0 (n = 11) cocoons per terrarium. Afterwards, they deposited a cocoon every 11 ± 6 days. After 120 days, the eleven earthworms deposited 78 cocoons, averaging 7.0 ± 4.0 cocoons per earthworm. Fecundity was low compared with figures obtained by Lavelle *et al*. (1987),

Fig. 1 Nest chamber built by *Pontoscolex corethrurus* to deposit their cocoons, showing walls coated with a layer of fine soil and mucus.

García and Fragoso (2003) and Bhattacharjee and Chaudhuri (2002). The low numbers of cocoons produced may be the result of low soil moisture in terraria (Lavelle *et al*. 1987).

P. corethrurus built spherical nest chambers (with a mean diameter of 5.97 ± 1.24 mm; n = 78) with the mouth, similar to chambers built during quiescence or diapauses (Evans and Guild 1947; Edwards and Bohlen 1996; Garnsey 1994; Jiménez *et al*. 1999, 2000; Lavelle and Spain 2001), where cocoons were deposited; nest-chamber walls were coated with a layer of fine soil and mucus (**Fig. 1**). Thus, our observations revealed that, unlike nest chambers built by some Lumbricidae (Ramisch and Graff 1985; Grigoropoulou *et al*. 2008), in the case of *P. corethrurus* nest chambers are not coated with cast. This suggests that *P. corethrurus* built nest chambers by: a) reducing water loss (desiccation) of cocoons, b) improving gas exchange between the nest chamber and the cocoon, c) protecting the cocoon against predation, and d) facilitating offspring emergence from the cocoon. Experiments should be carried to discriminate among these possibilities.

P. corethrurus deposited one spherical cocoon (Lavelle *et al*. 1987; Bhattacharjee and Chaudhuri 2002) and affixed it securely to the nest chamber by beating upon the inner chamber surface with the mouth, followed by suspending the cocoon in the air, with no contact with soil, through a number of mucus filaments attached to the nest wall (**Fig. 2**). Thus, the cocoon morphology (Edwards and Bohlen 1996; Lavelle and Spain 2001; Bhattacharjee and Chaudhuri 2002), which is typical of each species, may indicate the way cocoons are deposited inside incubation chambers.

Mean cocoon length was 3.64 ± 0.63 mm (n = 78), and mean fresh weight was 0.03 ± 0.04 g (n = 29). It is reported that cocoon weight, length and width varied with food quality and soil moisture (Lavelle *et al*. 1987; Bhattacharjee and Chaudhuri 2002; García *et al*. 2003). Mean cocoon incubation duration was 40 ± 9 days (n = 49), with one individual hatching per cocoon, reported mean cocoon incubation duration is 33.7 days (Barois *et al*. 1999; Bhattacharjee and Chaudhuri 2002). The long incubation period can be explained as a consequence of low soil moisture in terraria (Lavelle *et al*. 1987).

During incubation, *P. corethrurus* cocoons shifted in colour from off-white to pink. We observed that *P. corethrurus* adults frequently entered nest chambers for inner-wall repairing or cleaning, likely to avoid desiccation and eliminate microorganisms growing inside the nest. Around each

Fig. 2 Spherical cocoon deposited by *Pontoscolex corethrurus*; note the number of mucus filaments attached to chamber walls, suspending the cocoon to avoid contact with soil.

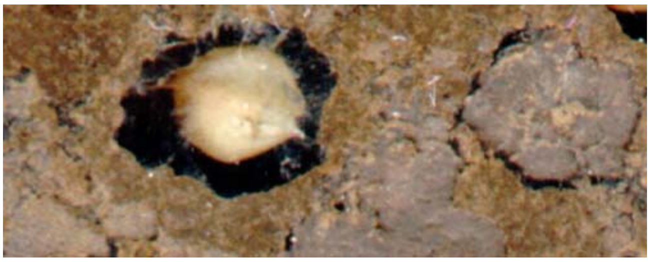

Fig. 3 Feeding chambers built by *Ponstoscolex corethrurus* around each nest chamber.

nest chamber, *P. corethrurus* consumed soil and built "feeding chambers" where cast were deposited (**Fig. 3**), returning frequently to these chambers to feed on either organisms (Tiunov and Scheu 1999; Bonkowski *et al*. 2000; Tiunov and Dobrovolskaya 2002; Curry and Schmidt 2007) or any other food source (Brown *et al*. 2004; Amador and Görres 2005; Amador *et al*. 2006; Le Bayon and Binet 2006) from casts. The mean weight of cast in the vicinity of nest chambers was 2.39 ± 1.05 g (n = 78). The average total weight of inner burrow cast in each terrarium was 17.35 ± 8.25 g.

During the observation period, once cocoons were deposited, *P. corethrurus* remained near nest chambers, without feeding in places other than nest or feeding chambers, except for building a new nest or feeding chamber. This behaviour suggests that, upon attaining sexual maturity, *P. corethrurus* stops depositing cast on the soil surface to place them inside feeding chambers. Several authors have reported that earthworms deposited casts on surfaces soil, displaying their activity as ecosystem engineering (Lavelle *et al*. 1987; Lavelle and Spain 2001; Topoliantz and Ponge 2005; Mariani *et al*. 2007). Hence, it is likely that surface cast are produced solely by juvenile earthworms.

After hatching, juvenile earthworms (with a mean fresh weight of 0.038 ± 0.01 g; n = 29) left nest chambers to feed on cast in feeding chambers. This behaviour suggests that, during the early growth and development stages, feeding chambers are built near nest chambers to supply newly hatched earthworms with quality food (microorganisms, nutrients and fine soil particles) until juvenile earthworms can feed on sources other than cast. Also, cast probably (Lavelle, pers. comm.) play a role in the insemination of parental gut microflora into the newly born earthworm gut. This is possible, given that cast are food sources for the development of other soil organisms (Brown *et al*. 2000; Brown and Doube 2004; Khomyakov *et al*. 2007) as well as for plant growth (Brown *et al*. 2004; Scheu 2003; Ortiz-Ceballos *et al*. 2007).

CONCLUSIONS

Our observations revealed that *P. corethrurus*: a) built nest chambers that are not coated with "cast" but with "fine soil" and mucus, b) a cocoon is suspended on mucus filaments, and c) both adults and juvenile worms seemingly feed on "casts" in feeding chambers.

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