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## Have spermatophores in *Eisenia fetida* (Oligochaeta, Lumbricidae) any reproductive role?

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### Summary

The presence of spermatophores is widespread in earthworms from the family Lumbricidae but their function remains unclear. We tested three functional hypotheses (sperm transfer, nuptial gift and copulatory plugs) to explain the presence of these structures in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae). None of the hypotheses tested were supported from our results and we found that spermatophores had no effect on the reproductive success of this earthworm species. Spermatophores appeared in all the matings that produced cocoons and hence may be used as reliable indicators of a recent copulation.

**Key words:** Earthworm reproduction, spermatophores, nuptial gift, copulatory plug, *Eisenia fetida*

### Introduction

In earthworms from the family Lumbricidae (Oligochaeta), spermatophores have often been described. Spermatophores are defined as capsules formed by glands in the neighbourhood of the male pore, filled with spermatozoa and placed on the body of the partner (Michaelsen 1926). In most lumbricids spermatophores appear frequently after mating. Nevertheless, the current knowledge about them is based only on anatomical criteria (Ljungström 1967; Bouché 1975; Gates 1978 a,b; Omodeo & Rota 1989). At present there are no data to support whether they are used in the fertilisation process, and their function, if any, remains unclear.

In this study we examined the function of spermatophores of *Eisenia fetida* (Savigny 1826), which is a common earthworm species with high incidence of

spermatophore presence. We tested three hypotheses on their function: (1) *Sperm transference*. In many animals spermatophores play an important role in sperm transfer (Mann 1984) and this is the function that has usually been proposed for them in Lumbricidae (Bouché 1975; Perel 1982; Edwards & Bohlen 1996). Spermatophores could be also an alternative method of sperm transfer, avoiding sperm digestion in the spermathecae, and fertilizing the ova during cocoon formation (Michiels 1998). (2) *Nuptial gift*. In many species of animals, males provide females with nutritional contributions during copulation (Thornhill & Alcock 1983). Sperm, packaged within a structure produced by specialized male accessory glands, represent a common form of nuptial gift transferred during mating in

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various animal taxa (Mann 1984). The sperm or other associated substances that are contained in spermatophores can be used as a resource to increase numbers of viability and quality of offspring (Gwynne 1984). (3) *Copulatory plug*. In hermaphrodite animals that fertilize one another, a conflict of interests could arise during the mating. Bateman's principle suggests that individuals copulate less to gain sperm to fertilize eggs but more to give sperm away (Charnov 1979). In this context, spermatophores could be structures that are produced by setal glands during mating and secreted to prevent sperm coming from the male pore of the partner to avoid insemination.

## Materials and Methods

Mature specimens of *Eisenia fetida* were obtained from a compost heap (Mos, Galicia, Spain) and from a stock culture maintained in the laboratory at a temperature of  $20 \pm 2^\circ\text{C}$  in cow manure.

### Spermatophores, mating and cocoon production

Numbers and position of spermatophores were examined under a dissecting microscope in forty single adult earthworms and in 56 mating earthworms once they had been separated, and both groups were collected from the compost heap. Spermatozoal morphology

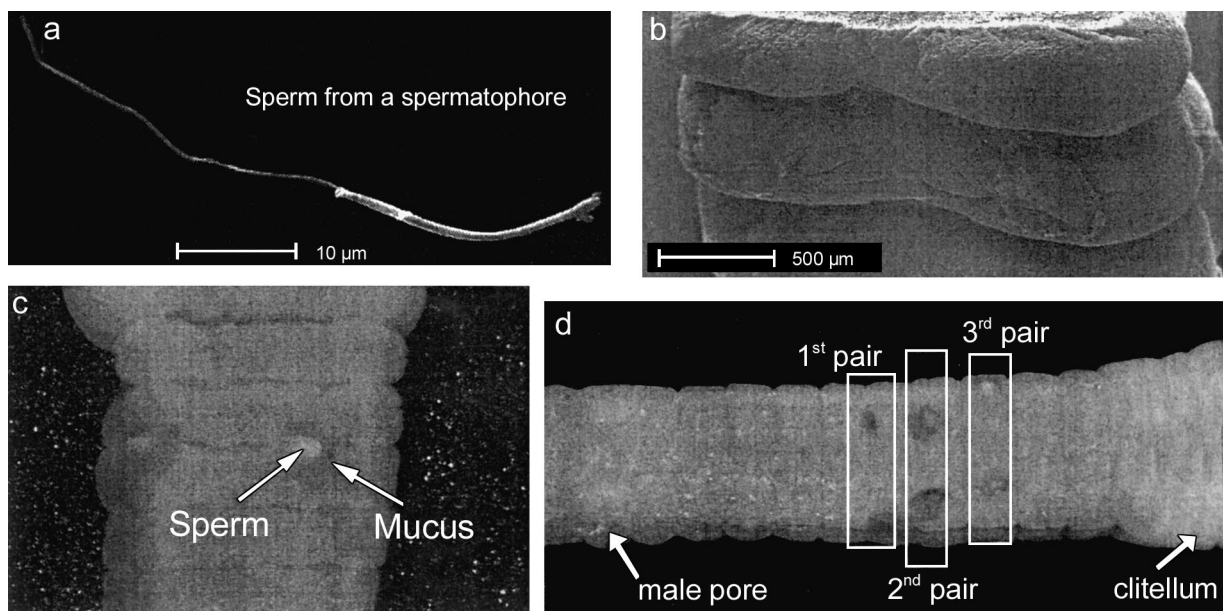
from the spermatophores was analysed under a Scanning Electron Microscopy and compared with typical sperm morphology in lumbricids (Jamieson 1992).

Twenty mature earthworms from a laboratory stock, each individual isolated from hatching, were paired for 17 h and the presence of spermatophores checked after this time. To determine if there is a relationship between the presence of spermatophores and sperm reception, earthworms were isolated until cocoon deposition. The copulatory plug hypothesis predicts that the presence of spermatophores will prevent further insemination. In addition, 44 earthworms possessing spermatophores were placed individually in Petri dishes and presence of spermatophores revised daily until the first cocoon was deposited to determine if spermatophores are included in the cocoons.

As we noticed that spermatophores disappeared before cocoons were deposited (see Results), they were marked with black ink in 15 earthworms from the stock and placed individually in Petri dishes lined with white paper to find them easily if they fell off the earthworm body.

### Experimental removal of spermatophores

To quantify possible effects on cocoon production, spermatophores were removed from 15 earthworms using tweezers. Another 15 earthworms were manipulated with tweezers but without removing the spermatophores. The numbers and weights of cocoons and



**Plate 1.** Shape, size and position of spermatophores in mature individuals of *Eisenia fetida*. (a) Scanning electron micrograph of sperm from spermatophores (b) Scanning electron micrograph of attached spermatophores (c) Detail of spermatophores (d) Location of spermatophores attached to the earthworm body wall

hatchlings were recorded. The nuptial gift hypothesis predicts that removing spermatophores would reduce reproduction resources and affect reproductive success. Fisher exact tests were used to determinate the statistical significance of the relationship between spermatophores presence and cocoon production. Differences in reproduction between control and removal group were analysed by t-tests.

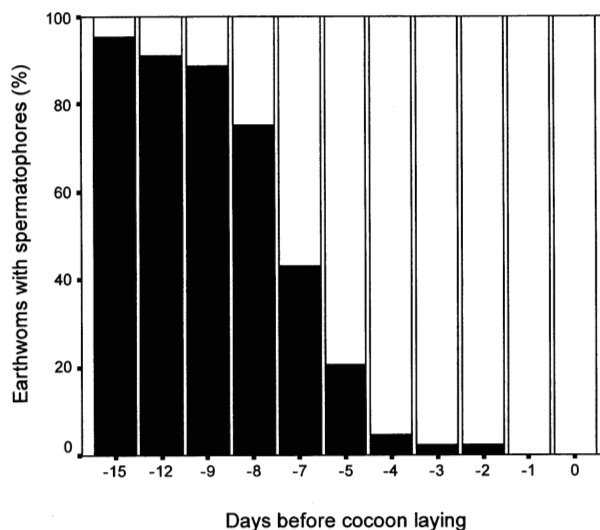
## Results

### Spermatophore description

Spermatophores are discs of mucus containing a drop of sperm that lie flat against the epidermis of the earthworm (Plate 1b–d). In all mature earthworms that were studied spermatophores were found between segments 21 and 24, inclusive. All spermatophores contained spermatozoa with the characteristic morphology of sperm from Lumbricidae (Plate 1a). We observed that the spermatophores from mating earthworms were brighter in colour than spermatophores from non-mating earthworms, probably due to oxidation of the mucus around the sperm droplet.

### Spermatophores, mating and cocoon production

We found that 86% of the earthworms had two spermatophores per segment and 14% had only one spermatophore immediately after mating. Only 2.3% of the earthworms had more than two spermatophores, and one individual had six spermatophores, the brightest placed posteriorly to the darker ones (see Plate 1d). After 17 hours together, spermatophores appeared in both partners in 40% of the mating couples and all of these earthworms produced cocoons (Fisher exact test,  $P < 0.001$ ). Spermatophores were not included in the cocoons during their formation; all earthworms lost their spermatophores before cocoon deposition. The first cocoon was laid from 1 to 15 days after the earthworms lost their spermatophores (Fig 1). We found all marked spermatophores in the substrate.



**Fig. 1.** Number of earthworms losing spermatophores with respect to cocoon formation. Earthworms were collected from a natural population

### Experimental removal of spermatophores

Cocoon production was not affected by removal of spermatophores. Cocoon viability was significantly more in earthworms with spermatophores removed. Reproductive success and cocoon and hatchling biomass did not differ significantly between the control and experimental groups (Table 1).

## Discussion

The results of this study can be summarized as follows: (1) Spermatophores contained sperm, (2) two spermatophores were found attached to the earthworm body after each mating, (3) only earthworms with spermatophores produced cocoons, (4) spermatophores always fell off the earthworm body before cocoon production, and (5) spermatophores did not affect reproductive success. These results do not fit any of the hypotheses proposed (sperm transfer,

**Table 1.** Effect of removal of spermatophores on the reproduction of *Eisenia fetida* (mean  $\pm$  S.E.). Cocoon production was recorded until the end of the oviposition period (2 months)

	Control	Experimental	t	p
No. cocoons per earthworm	15.73 $\pm$ 1.99	13.87 $\pm$ 2.10	0.645	0.524
Cocoon weight (mg)	166 $\pm$ 24	146 $\pm$ 25	0.564	0.577
Cocoon viability (%)	68.0 $\pm$ 4.3	80.5 $\pm$ 3.2	-2.334	0.027
No. hatchlings per earthworm	21.67 $\pm$ 3.34	25.87 $\pm$ 5.06	-0.693	0.494
Hatchling biomass (mg)	64 $\pm$ 12	67 $\pm$ 15	-0.175	0.862

nuptial gifts or copulatory plugs). Although Bouché (1975) suggested that spermatophores could be a device to ensure fertilization of eggs during cocoon deposition, our study showed that spermatophores in *E. fetida* appeared not to have any function in sperm transference because they were abandoned in the substrate before cocoon deposition.

There was no evidence supporting the idea that spermatophores are used as a nutritional "gift". Spermatophores were deposited on the soil, and they had no effect on reproductive success, but the presence of spermatophores was related to cocoon production (only earthworms with spermatophores laid cocoons). There was no evidence that spermatophores were used to avoid partner insemination, as the copulatory plug hypothesis predicted.

Thus our study has failed to elucidate the functional significance, if any, of the spermatophores and further work is needed to explain the presence of these structures. They could, for example, serve to force seminal fluid to emerge from the outer margin of the male pore and pass thence into the seminal groove as has been suggested by Grove (1925).

An interesting observation is that spermatophores were indicators of recent copula (in the last 3 days) and hence could be used as a tool to study copulation behaviour in *Eisenia fetida*. Thus, some earthworms that had more than two spermatophores showed that multiple mating occurs, behaviour not previously described for this species. All these data reveal our current lack of knowledge on the reproductive behaviour of earthworms in the family Lumbricidae. Spermatophores could contribute to our understanding of earthworm biology despite uncertainty in their function.

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