Pedo
biologia

Pedobiologia **47**, 530–534, 2003
© Urban & Fischer Verlag
http://www.urbanfischer.de/journals/pedo

The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002

Uniparental reproduction of *Eisenia fetida* and *E. andrei* (Oligochaeta: Lumbricidae): evidence of self-insemination

Jorge Domínguez*, Alberto Velando, Manuel Aira and Fernando Monroy

Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo E-36200, Spain

Submitted September 6, 2002 · Accepted May 6, 2003

Summary

This study investigated the copulatory behaviour of the lumbricid earthworm *Eisenia fetida* (Savigny, 1826) in a natural population, and the uniparental reproduction of *E. fetida* and *E. andrei* Bouché 1972, two closely-related species. Sperm transfer occurred in 61 % of the matings observed, of which 88.2 % showed bi-directional sperm transfer, 9.8 % unidirectional sperm transfer and one earthworm was self-inseminating. Although uniparental reproduction in earthworms has been reported by many authors, self-insemination has never been recorded before this study.

Moreover, specimens of the two species were reared in isolation from hatching to see if they were able to produce cocoons when clitellate. The percentage of earthworms that produced cocoons was significantly higher in *E. andrei* (33 %) than in *E. fetida* (3.5 %). Numbers of cocoon produced and the number of hatchlings per cocoon were also significantly higher in *E. andrei* than in *E. fetida*; the cocoon viability was similar in the two species. One of these isolated individuals of *E. fetida* that produced cocoons was dissected and sperm was found into the spermathecae, indicating that self-insemination could be responsible, to some extent, of uniparental reproduction. Since parthenogenesis has not been demonstrated in these species, further studies are needed to show if uniparental reproduction is due only to self-fertilization.

Key words: Self-fertilization, uniparental reproduction, copulation, *Eisenia fetida*, *Eisenia andrei*

Introduction

It is usually assumed that hermaphrodite animals are rarely self-fertilizing. Self-fertilization, the fusion of male and female gametes from a single genetic individual, is an extreme form of inbreeding that normally causes a reduction in fitness through inbreeding depression and a general reduction of heterozygosity and genetic diversity in the offspring (Charlesworth & Charlesworth 1987; Jarne & Charlesworth 1993). For this reason mechanisms of self-incompatibility have been developed in many species (Jarne & Charlesworth 1993).

In hermaphrodite animals with separate male and female gonads that are internally- inseminated, self-fertilization is unlikely anatomically, except in some groups that can self-copulate, such as Oligochaetae. André (1963) demonstrated that the spermatozoids of *E. fetida* were able to fertilize ova from the same earthworm. However, there is a considerable contro-

---

*E-mail corresponding author: jdguez@uvigo.es

versy about autofecundation in Lumbricidae. Some authors have found that earthworms reared in isolation were capable of producing cocoons (Gavrilov 1939; Hartenstein et al. 1980; Sims & Gerard 1985) and others determined that isolated individuals were not able to produce cocoons (Herland-Meewis 1954; Venter & Reinecke 1987; Cluzeau et al. 1992) or reported the production of infertile cocoons (Evans & Guild 1948; André & Davant 1972).

In this research we studied the copulatory behaviour of *Eisenia fetida* (Savigny, 1826) in a natural population and the uniparental reproduction of *E. fetida* and *E. andrei* Bouché 1972, two closely-related species of lumbricids.

Materials and Methods

Individuals of *Eisenia fetida* were obtained from a compost heap (Mos, Galicia, Spain) and individuals of *E. andrei* from a stock culture maintained in the laboratory at a temperature of 20 ± 2 °C using cow manure as a breeding medium.

Copulatory behaviour and cross-fertilization were studied in a natural population of *E. fetida* living in a compost heap. A total of 84 matings were observed, and the number of copulations with sperm transfer, whether reciprocal or not, and the number of self-fertilizing individuals recorded. Sperm transfer was recorded with the naked eye; bidirectional transfer was assessed as the presence of 4 drops of sperm in the spermathecal area of both earthworms; unidirectional sperm transfer involved the presence of sperm in the spermathecal area of only one of the partners (see Plate 1).

To compare uniparental reproduction of these two species, cocoons of *E. fetida* and *E. andrei* were placed in plastic Petri dishes, covered with distilled water and incubated at 25 °C. Hatchlings from the cocoons (n = 60 of *E. andrei* and n = 200 of *E. fetida*) were placed individually in plastic Petri dishes with cow manure. The earthworms were examined every 10 days to assess clitellum development and from day 50 they were examined every week to monitor overall cocoon production. The cocoons were removed from the culture medium, their weight measured and their viability studied. These procedures were followed over a period of three months. Fisher exact tests and *t*-tests were used to determinate the statistical significance of differences between viability and fecundity of cocoons in the two species.

Results

A total of 84 matings of *E. fetida* were studied in the compost heap. We found that sperm transfer occurred in 61 % of them, and 88.2 % of the matings had bidirectional sperm transfer, 9.8 % had unidirectional sperm transfer and one individual was self-copulating. This individual showed self constrictions of the slime tube formed, as occurs in a typical cross-mating; sperm pathway from the male pore to the clitellum and 4 drops of sperm over the dorsally placed apertures of the spermathecae were observed in the self-copulating individual (Plate 1).

The measured reproductive parameters of *E. fetida* and *E. andrei* reared in isolation are summarized in Fig. 1. The percentage of earthworms that produced cocoons was significantly higher in *E. andrei* (33 %)

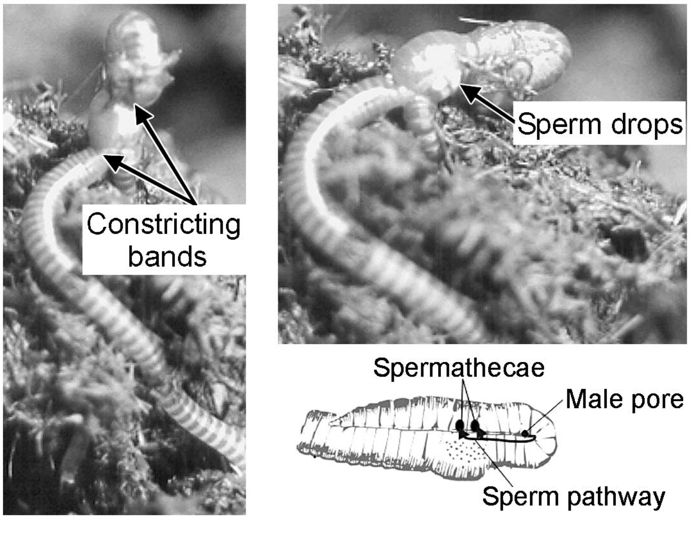

Plate 1. Self-insemination of *Eisenia fetida* in a natural high-density population. The scheme shows the process: the anterior part is folded over itself allowing contact of the spermathecal pores with the ventral zone of the clitellum; sperm flows from the male pore to the spermathecae as in a cross mating

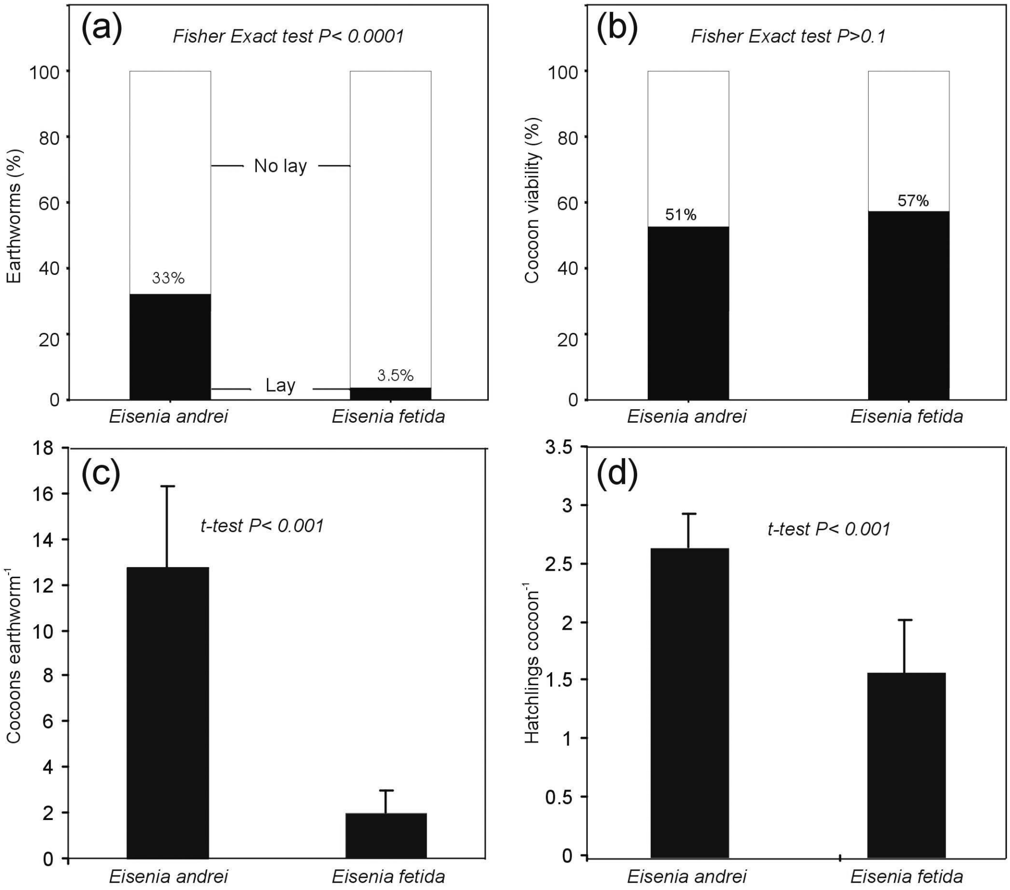

Fig. 1. Reproductive parameters of *E. fetida* and *E. andrei* reared in isolation. (a) Percentage of earthworms that lay cocoons. (b) Viability of the cocoons (%). (c) Number of cocoons produced per earthworm. (d) Number of hatchlings per cocoon

than in *E. fetida* (3.5 %). Number of cocoons produced and the numbers of hatchlings per cocoon were also significantly greater in *E. andrei* (12.8 ± 3.5 cocoons per earthworm and 2.6 ± 0.3 hatchlings per cocoon) than in *E. fetida* (2.0 ± 1.0 cocoons per earthworm and 1.6 ± 0.4 hatchlings per cocoon) and the cocoon viability was similar in the two species.

Discussion

André and Davant (1972) observed one specimen of *Dendrobaena rubida* (Savigny, 1826) with the anterior segments folded over themselves allowing contact of the spermathecal pores with the ventral zone of the clitellum and suggested that self-fertilization was possible. However, effective self-insemination had never been recorded before this study. According to Reynolds (1974) and Gates (1978) *E. fetida* must be considered to be an amphimictic species, i. e. with the normal mutual exchange of sperm between copulating individuals; Hartenstein et al. (1980) suggested that *E. fetida* was unable to self-fertilize, but possible "facultative" self-fertilization has been suggested by other authors (Sims & Gerard 1985). *E. andrei* has been postulated as an amphimictic species (Evans & Guild 1948; Venter & Reinecke 1987). However, we have observed one case of self-copulation in a high density natural population of *E. fetida*, and, as far as we know, this is the first clear evidence of self-sperm transfer in the Oligochaeta.

As other authors reported (e. g. Gavrilov 1939; Hartenstein et al. 1980; Sims & Gerard 1985; Domínguez et al. 1997; Nakagawa et al. 2002) we found that some earthworms reared in isolation from birth were capable of producing cocoons. One of these isolated individuals of *E. fetida* that produced cocoons was dissected and sperm was found into the spermathecae, indicating that self-insemination could be responsible, to some extent, of uniparental reproduction. Inbreeding depression, a major force selecting against

Table 1. Cross reproduction of *E. andrei* and *E. fetida*. Data represents a mean of data obtained from Elvira et al. (1996) and Domínguez et al. (1997, 2000). These experiments and the uniparental reproduction experiments (Fig. 1) were conducted with the same populations of earthworms and under similar conditions in our laboratory

SPECIES	Cocoons per earthworm	% Viability	Individuals per cocoon
E. andrei	17.4	75.8	3.43
E. fetida	12.3	88.3	3.75

selfish, can be calculated as 1-selfish fitness/outcrossing fitness; values > 0.5 promote the evolution of outbreeding (Jarne & Charlesworth 1993). Table 1 summarizes the reproductive parameters from cross-fertilization of the studied species in our laboratory under the same conditions. Using the numbers of hatchlings per earthworm as a fitness indicator, we found that the inbreeding depression was 0.44 for *E. andrei* and 0.93 for *E. fetida*. This agrees with the percentages of earthworms that produced cocoons by uniparental reproduction in our experiments, with *E. fetida* being selected against self-fertilization, but not *E. andrei*. The low probability of self-fertilization occurring in *E. fetida* could be a possible explanation for the results reported in other studies.

Although Nakagawa et al. (2002) reported the possibility of parthenogenesis in *E. fetida*, their conclusion was based only on records of two specimens producing cocoons before mating. There are long and confusing arguments in the literature attributing parthenogenesis to both species. Parthenogenesis has not been demonstrated in these species. Parthenogenetic taxa are characterized typically by high ploidy (Beukeboom et al. 1998). The stable coexistence between polyploidy and parthenogenesis in well separated phylogenetic taxa implies that interdependence and polyploidy is required for stable parthenogenesis (Soumalainen et al. 1987). Although some species of the genus *Eisenia* are polyploids (e. g. *E. nordenskioldi*, see Viktorov 1997), *E. andrei* and *E. fetida* are diploids (Muldal 1952), and hence real parthenogenesis is highly improbable. Further studies are needed to show if uniparental reproduction is due only to self-fertilization.

A possible explanation for self-fertilization in hermaphrodite animals living in high density populations could be: 1) adequate reproductive assurance in the case of dispersion, 2) reduction of negative costs of mating when the probability of finding a "good" partner is low and 3) reduction of the cost of mating when the probability of kin genotypes in the population is high.

References

André, F. (1963) Contribution à l´analyse expérimentale de la reproduction des lombriciens. Bulletin de Biologie de France et Belgique 97, 4–101.

Andre, F., Davant, N. (1972) L'autofecondation chez les lombriciens. Observation d'un cas d'autoinsemination chez *Dendrobaena rubida subrubicunda* Eisen. Séance, 12 du decembre, 725–728.

Beukeboom, L. W., Sharbel, T. F., Michiels, N. K. (1998) Reproductive modes, ploidy distribution, and supernumerary chromosome frequencies of the flatworm *Polycelis nigra* (Platyhelminthes: Tricladida). Hydrobiologia 383, 277–285.

Charlesworth, D., Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18, 237–268.

Cluzeau, D., Fayolle, L., Hubert, M. (1992) The adaptation value of reproductive strategy and mode in three epigeous earthworm species. Soil Biology and Biochemistry 29, 743–746.

Domínguez, J., Briones, M. J., Mato, S. (1997) Effect of the diet on growth and reproduction of *Eisenia andrei* (Oligochaeta, Lumbricidae). Pedobiologia 41, 566–576.

Domínguez, J., Edwards, C. A., Webster, M. (2000) Vermicomposting of sewage sludge: effect of bulking materials on the growth and reproduction of the earthworm *Eisenia andrei*. Pedobiologia 44, 24–32.

Elvira, C., Domínguez, J., Briones, M. J. (1996) Growth and reproduction of *Eisenia andrei* and *E. fetida* (Oligochaeta, Lumbricidae) in different organic residues. Pedobiologia 40, 377–384.

Evans, A. C., Guild, W. J. M. (1948) Studies on the relationships between earthworms and soil fertility IV. On the life cycles of some British Lumbricidae. Annals of Applied Biology 35, 471–483.

Gates, G. E. (1978) Contributions to a revision of the earthworm family Lumbricidae. XXII. The genus *Eisenia* in North America. Megadrilogica 3, 131–147.

Gavrilov, K. (1939) Sur la reproduction de *Eiseniella tetraedra* (Sav.) forma typica. Acta Zoologica Stockholm 20, 439–464.

Hartenstein, R., Neuhauser, E. F., Easton, E. G. (1980) Growth fecundity of F_2 *Eisenia fetida* derived from F_1S_1 both reared in isolation from birth. Megadrilogica 3, 185–187.

Herland-Meewis, H. (1954) Croissance et reproduction du lombricien *Eisenia fetida*. Annales Societe Royale Zoology Belgique 85, 119–151.

Jarne, P., Charlesworth, D. (1993) The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annual Review of Ecology and Systematics 24, 441–466.

Muldal, S. (1952) The chromosomes of the earthworms. I. The evolution of polyploidy. Heredity 6, 56–76.

Nakagawa, S., Bannister, T. D., Jensen, F. R., McLean, D. A., Waas, J. R. (2002) Relatedness does not affect the mating effort of *Eisenia fetida* Sav. (Oligochaeta) despite evidence for outbreeding depression. Biology and Fertility of Soils 35, 390–392.

Reynolds, J.W. (1974) Are oligochates really hermaphroditic amphimictic organisms? Biologist 56, 90–99.

Sims, R.W., Gerard, B.M. (1985) Earthworms. Synopses of the British Fauna 31. Linnean Society, London.

Soumalainen, E., Saura, A., Lokki, J. (1987) Cytology and evolution in parthenogenesis. CRC Press, Florida.

Venter, J.M., Reinecke, A.J. (1987) Can the commercial earthworm *Eisenia fetida* (Oligochaeta) reproduce parthenogenetically or by self fertilization? Revue d´Ecologie et Biologie du Sol 24, 157–170.

Viktorov, A.G. (1997) Diversity of polyploidy races in the family Lumbricidae. Soil Biology and Biochemistry 29, 217–221.