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Original article

Influence of size and partner preference on the female function of the earthworm *Eisenia andrei* (Oligochaeta, Lumbricidae)

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Abstract

Many aspects of the reproduction and mating behaviour of earthworms remain poorly understood. In this study, we focused on body size as a possible trait that influences earthworm reproduction and mating processes. *Eisenia andrei* is a simultaneously hermaphroditic animal with reciprocal insemination and many hermaphrodites are expected to mate not primarily to get their own eggs fertilized, but rather to get the opportunity to fertilize the eggs of their partners. We investigated whether *E. andrei* has a size-dependent sex allocation, i.e. if larger earthworms are more biased toward female allocation and produce more egg mass and whether *E. andrei* has a size-related mate choice by studying the relationship between mating delay and cocoon production. To test this, we compared cocoon production between pairs of earthworms of equal and different size. Mature individuals of *E. andrei* were classified in two size classes (small and large) and we performed a two-factorial experiment with earthworm size and the size of the partner as factors. After copulation, earthworms were isolated and thereafter their mass and the number of cocoons they produced were recorded weekly for 18 weeks. We found no evidence of size-dependent sex allocation and we found no effect of size-assortative mating on cocoon production. With respect to the differences in the time to mate, the mating delay seems to indicate the existence of some kind of mate choice, independent of the earthworm size. Those earthworms that were matched sooner laid many cocoons, but those that waited a long time to mate laid fewer cocoons. This effect was stronger in those individuals paired with large partners, suggesting that some large partners are more desired 'males' than others; the reason for this remain as an open question. In general, our results confirm that earthworms are able to discriminate their partners and adjust their breeding effort accordingly.

Keywords: Earthworms; Mate choice; Size-dependent sex allocation; *Eisenia andrei*

1. Introduction

In simultaneous hermaphroditic animals, the division of reproductive resources into male and female function is an important strategy for optimizing fitness [4]. In hermaphrodites as well as in gonochoristic species, the fecundity of the female function is normally limited by the amount of energy available for egg production, and the fecundity of the male function is normally limited by the number of eggs available [1,3]. Therefore simultaneous hermaphrodites are expected to mate not primarily to get their own eggs fertilized, but rather to get the opportunity to fertilize the eggs of their partners [7]. If two hermaphrodites meet, they are expected to be in a conflict over the amount of sperm each is allowed to give [6,9]. Because of this dilemma, they are expected to choose their mates [9] especially when individuals differ (e.g. in body size) [13].

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The earthworm *Eisenia andrei* is a simultaneously hermaphroditic animal with reciprocal insemination and as in many hermaphrodites female fecundity may be positively correlated with body size; hermaphroditic animals should prefer to donate sperm to larger partners and since they will tend to be rejected by partners that are larger than themselves, mating will mainly occur between equal-sized individuals. Here we studied whether *E. andrei* has a size-dependent sex allocation, i.e. if larger earthworms are more biased toward female allocation and produce more egg mass. We also investigated whether *E. andrei* has a post-copula reproductive adjustment of the female function according to the size of the partner.

2. Materials and methods

We studied the size-class distribution of earthworms (*Eisenia andrei*) in a field compost heap (Tomiño, Galicia, Spain) by collecting one hundred individuals. Then, two earthworm size classes were selected: large earthworms (larger than mean plus one standard error) and small (smaller than mean minus one standard error). To be sure that earthworms had not mate recently, we used earthworms without spermatophores (indicators of recent copula [11]). In order to analyze the earthworm female function (cocoon production) we designed a two-factorial experiment (earthworm size and partner size) where the earthworms were crossed in the combinations between the two size classes. Thus, the following experimental crosses were set up: large x large (16 earthworms), small × small (16 earthworms) and large x small (16 large earthworms and 16 small earthworms). These mating pairs were weighed and housed in plastic Petri dishes filled with vermicompost and fed with cow manure ad libitum. The dishes were maintained at 20°C and 90% relative humidity in a scientific incubator [5]. We measured the mating delay as the time (days) since we placed the earthworm pairs in the dishes until the apparition of spermatophores (evidence of copulation [11]). Then, earthworms were separated, weighed and placed individually into plastic Petri dishes and maintained as described above. The number of cocoons produced by each earthworm, determined by hand-sorting, was measured weekly for 18 weeks.

Two-way analysis of covariance (ANCOVA), with partner size (small and large), earthworm size (small and large) as factors and mating delay as covariate allowed determination of significant differences on cocoon production.

3. Results

Earthworm size did not affect cocoon production (Fig. 1, $F_{1,55} = 1.80$, $P = 0.18$). However, earthworms paired with small partners produced significantly more cocoons than those paired with large partners (Fig. 1, partner size, $F_{1,55} = 6.61$, $P = 0.013$). This effect was independent of the earthworm size (earthworm size x partner size, $F_{1,55} = 0.35$, $P = 0.55$).

The time to mate had a strong influence on cocoon production (Fig. 2, mating delay, $F_{1,55} = 10.87$, $P = 0.002$). Thus, cocoon productivity was higher in earthworms that mate earlier than in those that mate later; this effect tended to be stronger in earthworms mated with large partners than in those mated with small ones (partner size x mating delay, $F_{1,55} = 3.92$, $P = 0.053$).

4. Discussion

In many species of simultaneous hermaphrodites body size correlates with fecundity, and larger partners are preferred to small ones [7,12,15]. Since sperm exchange is usually reciprocal, small individuals may be rejected by larger partners resulting in size-assortative mating [13]. In our study we did not find any evidence of size-dependent sex allocation, i. e. larger earthworms did not lay more cocoons than smaller ones. This result is similar to a previous study in *Lum-*

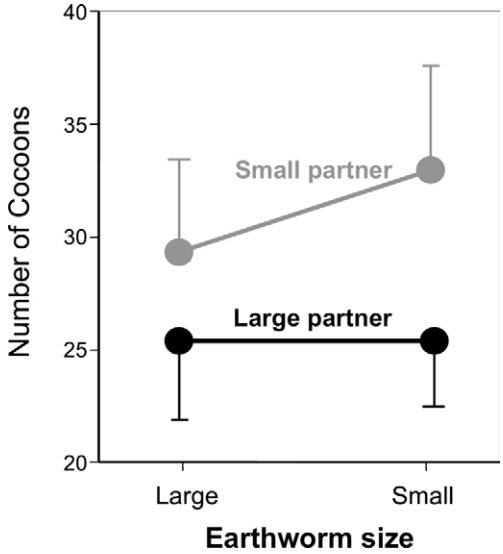

Fig. 1. Total number of cocoons (mean ± SE) produced by *E. andrei* during 18 weeks in the experimental crosses. Large and small earthworms were crossed with large (black circles) and small partners (grey circles) in a two-factorial experiment ($N = 16$ earthworms per group).

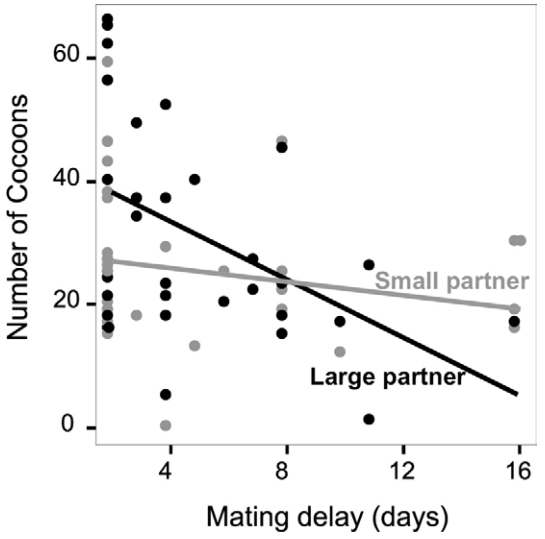

Fig. 2. Relationship between the total number of cocoons produced by *E. andrei* during 18 weeks after copulation) and mating delay (days since earthworm pairs were placed in the Petri dishes to copulation) according to the body size of the partner (large: black circles, $N = 32$; small: grey circles, $N = 32$).

bricus terrestris where large earthworms did not produce more cocoons [2], although it is argued that they tend to produce heavier cocoons and larger offspring [10]. We have no data to evaluate this hypothesis, but on the present evidence we cannot conclude that there is size-dependent reproductive effort in *E. andrei*.

As body size did not affect fecundity, larger partners are not necessary preferred to small ones. In fact we found no effects of size-assortative mating on cocoon production. Meyer and Bowman [8] suggested that *E. fetida* may adjust their sexual role according to its partner. Two recent studies showed that *Eisenia* spp. are able to adjust their breeding effort according to their desired partner; thus, *E. andrei* produced significantly less cocoons when crossed with *E. fetida*, investing less in hybrid cocoons due their inviability [5]. Other study showed that *E. andrei* due to their inviability adjusted its reproductive effort, measured as the number of cocoon produced, according to the degree of relatedness of its partner [14]. Thus, our results on size-assortative mating cannot be attributed to the unfeasibility of *E. andrei* to discriminate the partner and adjust the breeding effort accordingly.

With respect to the differences in the time to mate, the mating delay seems to indicate the existence of some kind of mate choice, independent of the earthworm size. Those earthworms that were matched sooner laid many cocoons, but those that waited a long time to mate laid fewer cocoons. This effect was stronger in those individuals paired with large partners, suggesting that some large partners are more desired "males" than others; the reason for this remain as an open question.

In general, our results confirm that earthworms are able to discriminate their partners and adjust their breeding effort accordingly.

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