

Original article

# Inbreeding and outbreeding reduces cocoon production in the earthworm *Eisenia andrei*

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## Abstract

Earthworms are animals with reciprocal insemination. *Eisenia andrei* Bouché, 1972 is a simultaneous hermaphroditic earthworm that lives in manure heaps at high densities, with low opportunities of dispersal, thus very close inbreeding is expected. As the negative effects of inbreeding and outbreeding may be severe, we studied whether *E. andrei* adjusts its breeding effort according to the degree of mate relatedness. To test this, we performed laboratory experiments in which earthworms were mated with their sibs and with non-sibs from the same population and non-sibs from a geographically isolated population. Inbreeding and outbreeding matings caused a strong reduction of cocoon production, especially in genetic lines with high reproductive rates. As far as we know, this is the first study that indicates reproductive adjustment in earthworms according to the genetic divergence of their partners. Optimal outbreeding should be considered a crucial point in the management of breeding populations for applied purposes.

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**Keywords:** Earthworms; Kin; Genetic divergence; Reproductive adjustment; *Eisenia andrei*; Cocoon

## 1. Introduction

High genetic similarity or relatedness of parents resulting from close inbreeding can contribute to declines in many wild populations [11,12]. Offspring from closely related parents commonly show reduced fitness [5], particularly under stressful conditions [3, 6]. This phenomenon has long been recognized and is referred to as inbreeding depression [5], and is thought to be one of the primary selective forces opposing the increase of deleterious recessive mutations [13]. Thus, avoidance of inbreeding has probably played a major

role in the evolution of dispersal and breeding behaviors [4,7]. On the other hand, extreme outcrossing may also decrease offspring fitness through the disruption of complexes of interacting genes that have jointly evolved, mixing of genomes adapted to different environments, or physical or physiological incompatibilities of partners from different population [18,19]. As the negative effects of inbreeding and outbreeding may be severe in many species, individuals would try to avoid inbreeding directly by kin recognition mate choice or indirectly, adjusting their breeding effort according to mate relatedness [1]. In nature, an optimum level of outbreeding has been rarely demonstrated in animal populations [2,14].

Earthworms are simultaneous hermaphroditic animals with reciprocal insemination [10]. *Eisenia andrei*

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Bouché, 1972 (*Oligochaeta*, *Lumbricidae*) lives in manure heaps at high densities, with low opportunities of dispersal [8], thus very close inbreeding is expected. Understanding the effect of inbreeding and outbreeding on the reproductive adjustment of this species can be a crucial point in the management of breeding populations for applied purposes. In this paper we determined whether *E. andrei* adjusts its breeding effort, measured as the number of cocoon produced, according to the degree of mate relatedness. To test this, we performed laboratory experiments in which earthworms were mated with their sibs and with non-sibs from the same population and no-sibs from a geographically isolated population.

## 2. Materials and methods

Two different natural populations of *E. andrei* were utilized in the experiments, Vigo (Northwestern Spain) and Madrid (Central Spain, 500 km apart). To ensure that the earthworms used were not storing spermatozoa from previous matings, juvenile specimens of the Vigo population, weighing 100–150 mg live weight, were individually placed in 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. The earthworms were raised until sexual maturity occurred, indicated by the presence of the clitellum, and then crosses were made between individuals of the same population. Hatchlings from five earthworms were raised individually, in order to obtain five families of four full-siblings (from the same earthworm mating). When siblings were mature, they were randomly assigned to three mating treatments nested by family (total number of crosses = 15): (1) inbreeding pair: mating two full-siblings, (2) intrapopulation pair: mating a sibling with a virgin earthworm from the same population (Vigo), (3) outbreeding pair: mating a sibling with a virgin earthworm from a distant population (Madrid). These mating couples were weighed and placed into plastic Petri dishes with vermicompost and cow manure for 7 days to ensure copulation. After this period, earthworms were separated and placed individually into the original plastic Petri dishes. Cocoon production of the pair earthworms, determined by hand-sorting, was measured weekly for 15 weeks.

The number of cocoons per pair was analyzed within full-sibling families by non-parametric repeated measures comparisons (Friedman test). In addition, we calculated the reduction in reproductive effort as the dif-

ference in cocoon production between intrapopulation and inbreeding matings (inbreeding reduction) and between intrapopulation and outbreeding matings (outbreeding reduction) within each genetic line (family of full-siblings).

## 3. Results

Within full-sibs family, there was a significant effect of pair relatedness degree (Friedman test,  $\chi^2 = 6.4$ ,  $P = 0.041$ ). Thus, inbreeding and outbreeding pairs produced, respectively, 30% and 19% fewer cocoons than the intrapopulation matings (Fig. 1). Earthworm families more affected by inbreeding (specially) and outbreeding (in some extent) matings were those more productive in the intrapopulation crosses (Fig. 2). Within sib-family, cocoon reduction by inbreeding and outbreeding matings were positively correlated (Fig. 2).

## 4. Discussion

Our results suggest that *E. andrei* adjusted its reproductive effort, measured as the number of cocoon produced, according with the degree of relatedness of its partner. Inbreeding and outbreeding matings caused a strong effect on cocoon production, especially in genetic lines with high reproductive rates. For iteroparous animals, such as earthworms, adjusting the investment in the egg to the mate's relatedness may be an adaptive strategy, since the offspring produced by inbreeding and outbreeding may have reduced their likelihood of success [17].

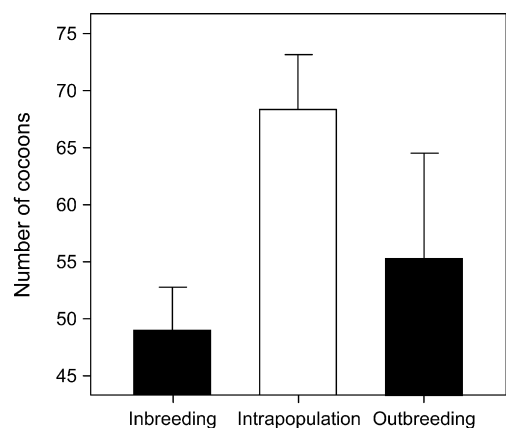
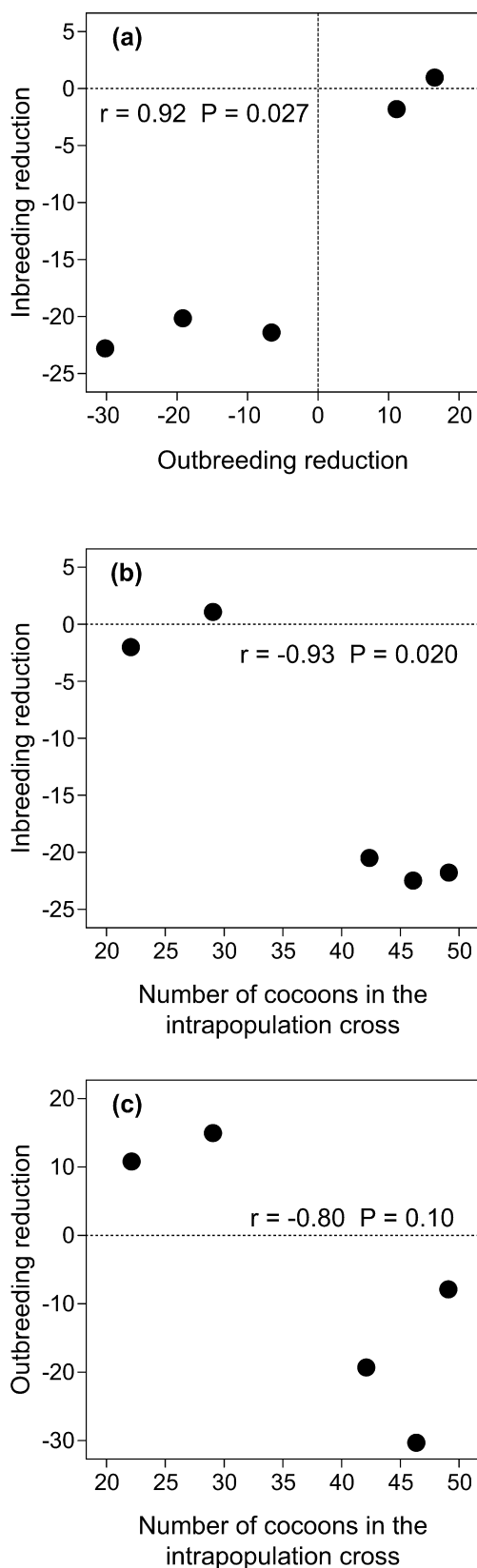


Fig. 1. Mean  $\pm$  S.E. of the number of cocoons produced by earthworm pairs in the inbreeding, outbreeding and intrapopulation crosses.



Life history theory predicts that iteroparous animals may maximize their lifetime reproductive success by balancing present and future reproduction, adjusting investment in a breeding event according to the likelihood of success [17]. In some species, it has been shown that individuals are able to change reproduction (number of eggs laid) when mate with ‘undesired’ male [16]. In *Eisenia* spp., reproduction is continuous during large part of its lifespan [20], and a reduction in the current reproductive investment should potentially have a positive effect on their residual reproductive value. In our study, the reduction in cocoon production suggests that earthworms are able to reduce their investment when are paired with detrimental mates. The high cocoon reduction in the genetic lines with high reproductive potential suggests that earthworm reproductive adjustment depends on their reproductive potential. Other studies also suggest that earthworm are able to adjust their breeding effort according with their mate. In fact, *E. andrei* from Vigo produced significantly less cocoons when crossed with *Eisenia fetida* than in the intrapopulation crosses, investing less in hybrid cocoons due their inviability [9].

In *E. fetida*, a close related species, a previous study that analyzed the inbreeding effect on reproductive adjustment did not find any effect on cocoon production [15]. Nevertheless, there are some differences with our study. First, the cited study could be not able to detect differences because the number of coons laid was recorded in a short period (2 weeks), in contrast with the long period (15 weeks) in this study. Second, we analyzed the cocoon production within sib-family taken to account the genetic variability in the responses. Finally, differences may be due to species-specific responses to within pair genetic relatedness.

As far as we know, this is the first study that indicates reproductive adjustment in earthworms according to the genetic divergence of their partners. Inbreeding and outbreeding crossed reduced the number of cocoons suggesting an optimal outbreeding degree.

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Fig. 2. Relationships between the number of cocoons per earthworm in the intrapopulation crosses, inbreeding and outbreeding reduction (measured as the difference in cocoon production regard to the intrapopulation mating) within each genetic line (family of full-siblings).

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