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Are Eisenia fetida (Savigny, 1826) and Eisenia andrei Bouché (1972) (Oligochaeta, Lumbricidae) different biological species?

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Summary

Two closely related earthworm species, Eisenia fetida (Savigny, 1826) and Eisenia andrei Bouché (I.N.R.A. Publ. Ann. Zool. Ecol. Anim. (no. hors-serie) 72(2) (1972) 671pp.) were analysed for reproductive isolation in laboratory experiments. The problem of their taxonomic status remains unresolved and moreover in much of the current literature both species are termed indiscriminately as E. fetida or E. foetida, and it is often not clear which of the two species is being referred to. Mature virgin individuals of different populations of E. andrei and E. fetida were housed in couples for a week. After copulation, earthworms were isolated and thereafter their mass, the number of cocoons they produced, the hatching success and the number of hatchlings per cocoon were recorded weekly for 15 weeks. The interspecific and intraspecific crosses confirmed that there is reproductive isolation between E. fetida and E. andrei; they can therefore be considered distinct biological species with different life histories. This evidence implies some important considerations; in vermiculture or vermicomposting E. andrei is more recommended since its growth and reproduction rates are higher. In studies on ecotoxicology, it is not possible to assume that contaminants will have the same effect on the two species, since their responses to stress factors could be different. The existence of postcopula but not precopula isolation in sympatric populations clearly affects the population dynamics by reducing the individual's fitness. For this reason, in applied aspects it is important keep the two species separated.

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Introduction

The importance of taxonomy is clearly recognized by the majority of scientists and without reliable taxonomy, ecological studies are irrelevant. In the case of lumbricid earthworms, taxonomic identification is often difficult because of the lack of stable and easy to handle diagnostic characters (Pop et al., 2003).

The closely related species Eisenia fetida (Savigny, 1826) and Eisenia andrei Bouché, 1972 (Oligochaeta, Lumbricidae) are those most commonly used for management of organic wastes, and also in ecotoxicology, physiology and genetics studies, mainly because they are ubiquitous with a world-wide distribution, their life cycles are short, they have a wide temperature and moisture tolerance range and they are resilient earthworms which can be readily handled (Domínguez, 2004). They were first described as different morphotypes of E. fetida according to differences in body pigmentation (André, 1963), and Bouché (1972) gave them sub specific status, naming them E. foetida foetida and E. foetida unicolour. Although now many authors accept E. foetida and E. andrei as different species, most older literature and even abundant current literature refer to these species collectively as E. fetida or E. foetida, an illegal emendation of the original E. fetida (Sims, 1983; Easton, 1983).

E. fetida corresponds to the striped or banded morph, with the area around the intersegmental groove having no pigmentation and appearing pale or yellow; hence, its common names of "brandling" or "tiger" earthworm; whereas E. andrei, the common "red" worm, corresponds to the uniformly reddish morph. Aside from the differences in pigmentation, the two species are morphologically similar (Sims and Gerard, 1985; Reinecke and Viljoen, 1991) and their requirements, overall reproductive performances and life cycles do not differ significantly, although growth rate and cocoon production are higher in E. andrei (Elvira et al., 1996). Roch et al. (1980) and Valembois et al. (1982) found important biochemical differences between both species and they suggested that E. andrei could have derived from E. fetida by the loss of some alleles. Fixed allelic differences exist at the mannose phosphate isomerase (Mpi) (Henry, 1999), the phosphoglucomutase (Pgm) loci, and the alanyl-amino peptidase (Aap) locus (Jaenike, 1982). Furthermore, E. fetida is polymorphic at the glucose phosphate isomerase (Gpi) locus, whereas E. andrei is monomorphic (Jaenike, 1982). Albani et al. (2003) found that E. andrei and E. fetida have specific fluorescence fingerprints and affirm that the two species do not metabolize the same types of molecules.

The life cycles of *E. fetida* and *E. andrei* and their population biology have been investigated by several authors (Graff, 1974; Watanabe and Tsukamoto, 1976; Hartenstein et al., 1979; Kaplan et al., 1980; Edwards, 1988; Venter and Reinecke, 1988; Reinecke and Viljoen, 1990, 1991; Elvira et al., 1996; Domínguez and Edwards, 1997; Domínguez et al., 1997; Domínguez et al., 2000) and the literature has been recently summarized by Domínguez (2004). The problem of the taxonomic status of the complex E. fetida/andrei remains unresolved and moreover, in much of the current literature, both species are termed indiscriminately as E. fetida, and it is not clear which of the two species is being referred to. Thus for example *E. foetida* is the recommended species in standard toxicity bioassays (OECD, 1984; Commission of the European Communities, 1983) and these procedures say ".... Eisenia foetida exists in two races which some taxonomists have separated into species (Bouche, 1972). These are morphologically similar but one, Eisenia foetida foetida, has typically transverse striping or banding on the segments and the other, Eisenia foetida andrei, lacks this and has a variegated reddish colour. Where possible Eisenia foetida andrei should be used..."

The two species are syntopic, commonly living in mixed colonies in dung and compost heaps and therefore hybridization could be possible. Hybridization between populations or species can have detrimental effect on fitness and strong effects on population dynamics in mixed colonies. In this case, reproductive isolation can be expected and it can be prezygotic, i.e. due to reproductive incompatibility or postzygotic, i.e. leading to a reduction in viability of the hybrid offspring.

Our objective in the current investigation was to determine if *E. andrei* and *E. fetida* are different biological species, i.e. if they are reproductively isolated. We present the results of laboratory experiments to test for pre- and postzygotic reproductive barriers by comparing cocoon and hatchling production of the two species in experimental inter-specific crosses. We also studied the intra-specific variability by comparing cocoon and hatchling production of *E. andrei* in experimental crosses between individuals from geographically isolated populations.

Materials and methods

Four different populations of earthworms (one *E. fetida* and three *E. andrei*) were utilized in the

experiments. Individuals of *E. fetida* were obtained from a compost heap (Mos, Galicia, Spain) and individuals of *E. andrei* from three separated populations (Vigo, Northwestern Spain; Madrid, Central Spain, 500 km apart, and Juiz de Fora, Brazil). To ensure that the earthworms used were not storing spermatozoa from previous matings, juvenile specimens of the four populations, 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 some combinations of the four earthworm populations (total number of population crosses = 7; Table 1). Mating partners were assigned haphazardly based on the individual identification numbers (total number of crosses = 32) and the weight of both partners in each cross was similar. These mating couples were weighed and placed into plastic Petri dishes with vermicompost and cow manure for 7 days. After this period, earthworms were weighed, separated and placed individually into the original plastic Petri dishes. Cocoon production of the earthworms, determined by hand-sorting was measured weekly for 15 weeks. All cocoons were placed among dampened cotton in microplate wells to enable the measurement of incubation time, viability rate and number of hatchlings per cocoon.

Generalized linear models (GLM; Wedderburn, 1974; McCullagh and Nelder, 1989) were performed to determine significant differences between reproduction parameters in the different crosses. The link function and error distribution in the GLMs were applied taking into account the presumed error distribution of the data and selecting those that minimized the deviance in the model (McCullagh and Nelder, 1989; Crawley, 1993; Herrera, 2000). Thus, Gaussian errors and identity link were

Table 1. Number of earthworms in the experimental crosses for one population of *E. fetida* (Vigo) and three populations of *E. andrei* (Vigo, Madrid and Brazil)

ida E. andre	
(Vigo)	(Madrid)
8 10 —	10 _
	8

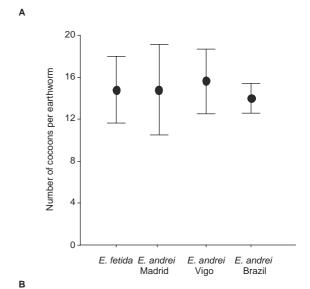
^{-,} Crosses not performed.

selected for the analysis of cocoon and hatchling production and binomial errors and logit link for the analysis of cocoon viability.

Results

There were no significant differences in the cocoon production of *E. fetida* in the four experimental crosses (Fig. 1A; GLM, $F_{3,20} = 0.26$, P > 0.5), but there were significant differences in cocoon viability (GLM, $F_{3,20} = 24.03$, P < 0.0001); thus, in *E. fetida*, only the intraspecific crosses produced viable cocoons (Fig. 1B).

In *E. andrei*, there were no significant differences in the cocoon production of the two studied populations (Vigo and Madrid) (Fig. 2A; GLM,



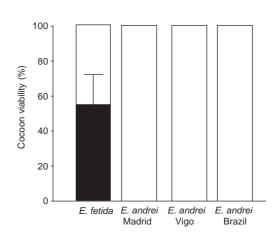
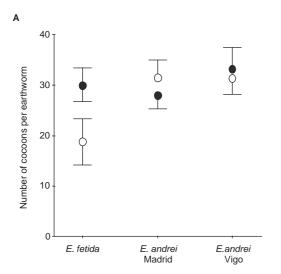


Figure 1. Mean ± SE of (A) number of cocoons laid during 15 weeks by *E. fetida* and (B) their viability, after mating with *E. fetida* and three populations of *E. andrei*. Note that no fertile cocoons were obtained in the crosses with *E. andrei*.

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 $F_{1,32}=1.18$, P=0.29;); and there were nor effect of the mating cross (crossed with E. fetida, E. andrei [Vigo], E. andrei [Madrid]) (Fig. 2A; GLM, $F_{2,32}=2.26$, P=0.12). The population of E. andrei from Vigo produced significantly less cocoons when crossed with E. fetida than in the intrapopulation crosses (t=2.34 g.l. =11 P=0.039). Nevertheless, the interaction between population and mating cross was not significant (population x cross; GLM, $F_{2,32}=2.01$, P=0.15). In E. andrei, only the intraspecific crosses produced viable cocoons, thus cocoon viability was significantly different depending on the cross (Fig. 2B; GLM, $F_{2,34}=41.08$, P<0.0001) but not on the population



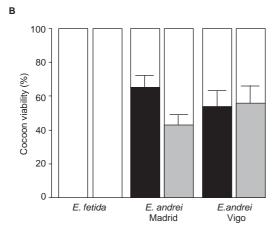


Figure 2. (A) Number of cocoons laid during 15 weeks by two populations of E. andrei from Madrid (filled circles) and Vigo (open circles) in the crosses with E. fetida and with the two populations of E. andrei (B) Cocoon viability of the two populations of E. andrei (Madrid (black bars) and Vigo (grey bars) in the crosses with E. fetida and two populations of E. andrei. Values are means \pm SE. Note that there were no fertile cocoons in the crosses with E. andrei.

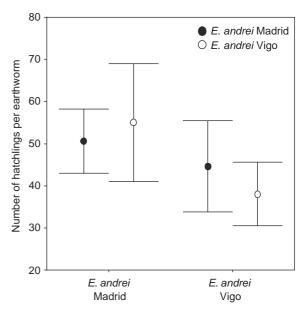


Figure 3. Number of hatchlings per earthworm produced during 15 weeks by two populations of E. andrei from Madrid (filled circles) and Vigo (open circles) in the intrapopulation crosses. Values are means \pm SE.

(Fig. 2B; GLM, $F_{1,36} = 2.08$, P = 0.16). The interaction between population and cross was not significant (GLM, $F_{2,32} = 1.21$, P = 0.31).

The number of hatchlings per cocoon was significantly higher in *E. andrei* (2.75 ± 0.13) than in *E. fetida* (2.11 ± 0.24) .

The total number of hatchlings produced by *E. andrei* did not differ between populations (Madrid, Vigo) and crosses (intra and inter population) (Fig. 3, GLM, Population: $F_{1,24} = 1.38$, P = 0.25; Cross: $F_{1,24} = 0.01$, P > 0.5 and for the interaction between population and cross neither was significant ($F_{1,24} = 0.32$, P > 0.5).

Discussion

Our laboratory experiments showed that *E. fetida* and *E. andrei* are reproductively isolated because no viable offspring was produced when crossed, so they should be considered as two different species according to the biological definition of species (Mayr, 1940) corroborating the hypothesis advanced by Jaenike (1982). Our findings differ from those found by André (1963) and Sheppard (1988). Andre created chimeras using surgery such that the male and female gonads in an individual came from the two species. By crossing such chimeras he found that male *E. fetida* and female *E. andrei* gametes did not produce viable offspring and that the

reciprocal cross produced hybrid offspring; these hybrids showed a banding pattern intermediate to that of their parents and laid cocoons, although these were all infertile. Sheppard (1988) found hatchlings in crosses between E. fetida and E. andrei, but stated that the offspring could be the result of hybridization, self-insemination or "facilitated self-fertilization", so this evidence should be taken with caution. McElroy and Diehl (2001) did not obtain interspecific hybrids and reported that Nei's (1978) genetic distance calculations based on allozyme frequencies within each population suggest that E. fetida and E. andrei are genetically distinct species, whereas within each species the populations are genetically similar. In addition, the reproductive isolation between E. fetida and E. andrei can not be attributed to exogamy depression (Dobzhansky, 1948; Templeton, 1986; Lynch, 1991) since there were no differences in hatchling numbers in the inter population crosses of *E. andrei* (see Fig. 3), i.e. exogamy did not reduce the fitness of E. andrei. The idea of the existence of a single, polymorphic species of *E. fetida* is rejected and we suggest that the status of 'good-species' (Mallet, 1995) can be applied to the taxa analysed, since both phenotypes are well discriminated.

Our results indicate that the isolation between *E. fetida* and *E. andrei* is postcopula, probably postzygotic, without efficient mechanisms to avoid inter specific matings. In fact, we found that the number of cocoons produced was similar in the intra and inter specific crosses of the two species, indicating that no precopula mechanism prevented mating and cocoon production.

Postcopula and postzygotic isolating mechanisms prevent the development of the zygote and in our study result in hybrid inviability in which hybrid individuals do not survive and not hybrid sterility in which hybrids are unable to reproduce. Postzygotic isolation in E. fetida and E. andrei can be characterized as intrinsic, since it depends on developmental problems that are relatively independent of the environment (Turelli et al., 2001). Independently of the type of isolation, it seems clear that this is incipient, since it has a deep effect on the fitness of the individuals; the two species mate and produce cocoons although these cocoons are sterile and the apparition of mechanisms preventing mating and cocoon production are expected in order to avoid unnecessary energy and time costs. In fact, in our experiments the population of E. andrei from Vigo produced significantly less cocoons when crossed with E. fetida than in the intrapopulation crosses, suggesting that the population of E. andrei from Vigo invest less in hybrid cocoons due their inviability. This could be indicative of the development of some prezygotic isolating mechanisms that avoids investment of resources in sterile matings. One possible explanation of the difference in cocoon production of the two populations of *E. andrei* when crossed with *E. fetida* could be the ecological differences between the two populations; the population from Madrid came from a commercial facility whereas the population from Vigo came from a "natural" manure heap and it could have been in touch with individuals of other species. For instance, females in the hybridogenic complex of *Rana lessonae* – *Rana esculenta* change their behaviour (number of eggs laid) if amplexed by the 'undesired' male (Rever et al., 1999).

Regarding the type of speciation, there is not enough information to know if it is sympatric or allopatric with secondary contact. In the latter case, the genomes of the two isolates could have evolved such that they have become incompatible or, if not completely incompatible, isolation mechanisms might be reinforced, leading to speciation by reinforcement (Johannesson, 2001).

In our experiments, *E. andrei* produced significantly more cocoons than *E. fetida* (Table 2) and cocoon viability was similar in both species and this is in agreement with the results obtained by Haimi (1990), Reinecke and Viljoen (1991) and Elvira et al. (1996); however, Sheppard (1988) reported similar cocoon production for both species (see Table 3). The number of hatchlings per cocoon was higher in *E. fetida* than in *E. andrei* and this is in agreement with some previous studies although this parameter is highly variable (Table 3).

Given the morphological and ecological similarity between these species, it is likely that competition plays a crucial role in their partially exclusive distribution.

Distribution maps of these species in Galicia published by several authors indicate a non-overlapping distribution (Souto and Mascato, 1993; Monroy et al., 2003), but *E. fetida* is clearly more abundant in natural environments. However,

Table 2. Cocoon production and cocoon viability (mean and standard error) of *E. fetida* and *E. andrei* in the intraspecific crosses

	Experimental crosses		
	E. fetida × E. fetida	E. andrei × E. andrei	
Cocoon production Cocoon viability (%)	19.7 (3.2) 61.2 (18)	30.5 (1.6)* 56.8 (4)	

^{*}P<0.001.

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Table 3.	Comparison of the	reproductive p	potential of E .	fetida and E.	andrei (Oligochae	ta, Lumbricidae). The
experimen	ts were conducted w	ith different po	opulations of ea	rthworms and	under different la	oratory conditions

Authors	Specie	Ta ¹	No cocoons ew ⁻¹ week ⁻¹	No hatchlings cocoon ⁻¹	Hatching success (%)	Food
Sheppard (1988)	E. fetida	24°	1.8	4.55	82.2	Cow manure
	E. andrei	24 °	1.34 ± 0.23	2.86	73.5	Cow manure
Haimi (1990)	E. fetida	20 °	1.8 ± 0.7	3.4 <u>+</u> 1.5	77.5	Various
	E. andrei	20 °	3.1 ± 0.1	1.9 <u>+</u> 0.5	85	Various
Reinecke and	E. fetida	25°	0.4	2.9 ± 0.2	89.2	Cow gut content
Viljoen (1991)	E. andrei	25°	0.67	4.4 <u>+</u> 0.2	90.5	Cow gut content
Elvira et al. (1996)	E. fetida	20 °	1.33	3.75	88.3	Cow manure
	E. andrei	20 °	1.47	3.06	88.1	Cow manure
This study	E. fetida	20 °	1.79 ± 0.3	2.11 ± 0.2	61.2	Cow manure
-	E. andrei	20 °	2.8 ± 0.1	2.85 ± 0.1	56.8	Cow manure

 $T_{\underline{a}}^{1}$, incubation temperature.

E. andrei is the predominant species in commercial exploitations of vermiculture and vermicomposting. Several observations suggest that E. andrei is spreading in NW Spain. Regarding competition, in previous experiments in our lab with mixed cultures of both species (see Elvira et al., 1996) we found that both species compete and E. andrei is dominant when food is abundant, whereas E. fetida is dominant when food is scarce; the results obtained in those experiments pointed to E. andrei being a more extreme r strategist than E. fetida as evidenced by more rapid growth and reproduction.

In conclusion, E. fetida and E. andrei are two different biological species with different life histories and this evidence implies some important considerations. In vermiculture and vermicomposting *E. andrei* is more recommended since its growth and reproduction rates are higher. In studies on ecotoxicology, although both species have quite similar ecological and probably physiological characteristics, it is not possible to assume that contaminants will have the same effect on the two species, since their responses to stress factors could be different. The existence of postcopula but not precopula isolation in sympatric populations clearly affects the population dynamics by reducing the individual's fitness. For this reason, in applied aspects it is important keep the two species separated.

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