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Growth and reproduction of *Eisenia andrei* and *E. fetida* (Oligochaeta, Lumbricidae) in different organic residues

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Summary. A comparative study of *E. andrei* and *E. fetida* growing in different organic residues was carried out to determine whether the population dynamics are substantially different in these two taxonomically close related species. Growth rates in three of the four different residues tested (paper pulp mill sludge, domestic refuse, cow manure and rabbit manure) were similar. *E. andrei* needed less time for clitellum development and cocoon production than *E. fetida* and this provides an important competitive advantage in F1 recruitment for the latter species. Cocoon size, hatch period and number of hatchlings per cocoon were slightly in *E. fetida*.
When both species were bred together, no negative effects on growth and reproduction were detected.

Key words: *Eisenia andrei, Eisenia fetida*, growth rates, cocoon production, food source

Introduction

The taxonomic status of *Eisenia andrei*, Bouché 1972, and *Eisenia fetida* (Savigny 1826) was confirmed by Jaenike (1982), employing electrophoretic techniques, who found three loci without common alelles, but until recently they have usually been considered as subspecies or varieties according to their different body pigmentation. André (1963) described *Eisenia foetida* form *typica*, with a characteristic striped pattern and *Eisenia foetida* form *unicolor* with a uniform reddish colour. Bouché (1972) considered that the term *unicolor* had a low systematic value, specially in the case of specimens kept in preservation liquids during long periods and for this reason, he designated these forms *Eisenia foetida foetida* and *Eisenia foetida andrei*.
On the basis of the biological definition of species, André was the first to demonstrate the specific status of these two forms by recording signs of reproductive isolation between them. He created chimeras, by means of surgery, such that the male and female gonads in an individual chimera proceeded from different species and the interbreeding resulted in infertile offspring. He was not prepared to call them "species" and it was necessary to wait for biochemical studies in the '80s (Roch et al. 1980, Jaenike 1982, Valembois et al. 1982, Engelstad & Stenersen 1991) to give them the status of separate species.
Both species are commonly employed in stabilizing organic materials and although most authors now accept them as different species (Sims & Gerard 1985, Sheppard 1988), it can be clearly seen that, in the literature, both species are indiscriminately termed *E. fetida* or *E. foetida*. One possible explanation is that except for pigmentation, the species are indistinguishable, showing similar body length and segment number as well as showing resemblance in the shape of the clitellum and tubercula pubertatis.

In order to obtain more details about some aspects of the reproductive biology of *E. fetida* and *E. andrei*, and to supply new criteria for their charaterization, a study of their growth and reproduction rates, mortality, and the biometry of their cocoons and hatchlings in four different food sources was carried out.

Materials and Methods

Growth and reproduction in different organic residues

8 immature specimens of both species (0.1 – 0.2 g fresh weight) were reared in the following organic sources: sludges proceeding from a paper pulp factory (Empresa Nacional de Celulosas, Pontevedra, Spain), organic fraction of domestic refuse, cow manure and rabbit manure. Moisture content was adjusted to 80% (wet weight) and the temperature ranged between 20 and 25 °C during the whole experiment.
600 cm^3 plastic containers (9 cm diameter and 10 cm high) were filled with each substrate to a height of 6 cm. Two replicates per treatment were established and no supplementary food was added during the whole experimental period.
The individual weights and clitellum development were monitored weekly and the cocoons removed for cocoon production assessment.

Pure and mixed cultures of E. fetida and E. andrei

Three populations were under study: a pure population of *E. andrei* (Ea), a pure population of *E. fetida* (Ef) and a mixed population with both species growing together (Ea* and Ef*). Pure populations were founded from the incubation (20 °C and darkness) of juveniles hatched from 50 cocoons, and the mixed population from 25 cocoons of each species. The newly hatched individuals were introduced into 5000 cm^3 containers (16 cm diameter and 25 cm high) with cow manure as the food source and supplementary manure was added regularly in order to avoid growth limitation.
After 30 days, biomass, number of clitellate specimens and cocoon production were recorded every two weeks for 160 days. Additionally, a subsample of 50 cocoons was taken to determine two biometric parameters (length and width), viability as percentage of hatching, hatching time, number of hatchlings per cocoon, cocoon biomass and depth of cocoon-laying by recording the numbers in two layers 0 – 4 and 4 – 8 cm. The linear dimensions and weight of 50 newly-hatched worms were also measured.
Finally, a study of offspring derived from the mixed population was performed to assess the dominance relationships between both species over time, determining the number of individuals and their biomass after 80, 95 and 110 days.

Statistical analyses

ANOVA and Fisher's LSD test allowed determination of significant differences between growth rates of both species in the four treatments and between pure and mixed cultures growing in the same substrate.

Results

Growth and reproduction in different organic residues

In general, growth rates and cocoon production were higher for *E. andrei* than *E. fetida* in all the treatments studied and the magnitude of these differences was clearly dependent on substrate nature (Fig. 1).
Although paper pulp mill sludge (Fig. 1a) did not allow growth, mortality was nil and weight losses were very low (−0.11 mg/worm/day for *E. andrei* and −0.28 mg/worm/day for *E. fetida*). Moreover, *E. andrei* lost significantly less weight ($p < 0.01$) than *E. fetida* during the whole experimental period.

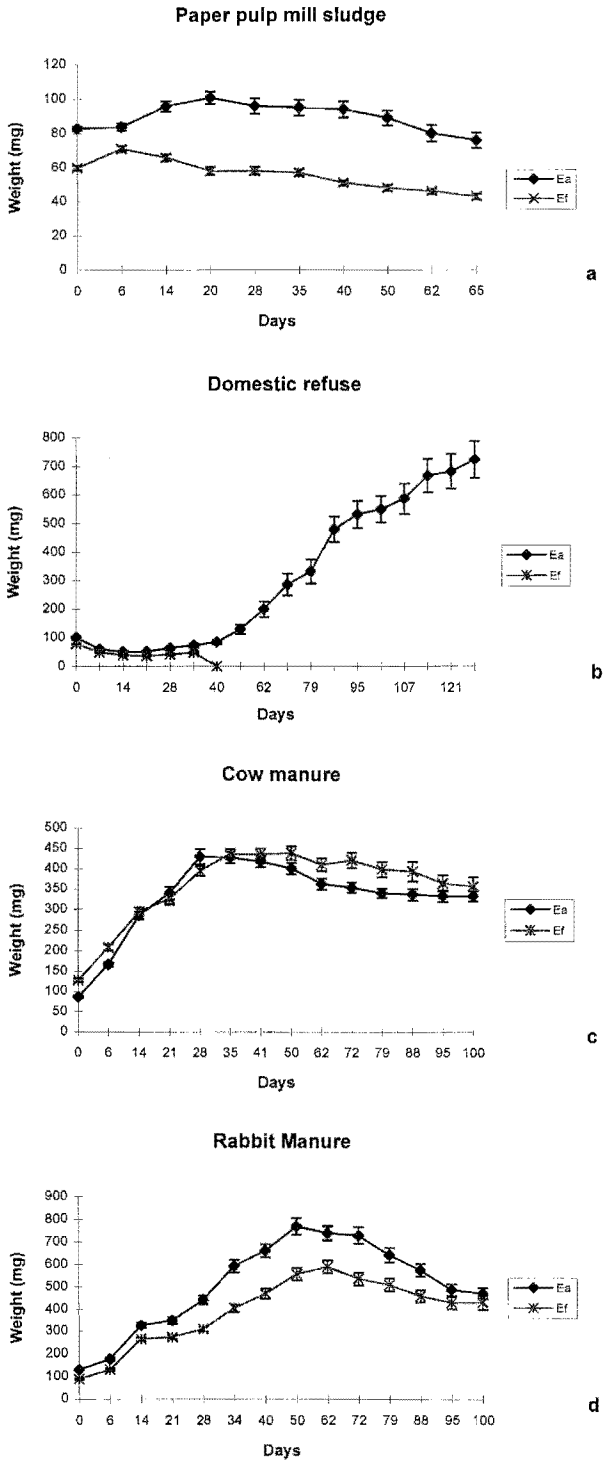

Fig. 1. Growth of *E. andrei* and *E. fetida* in four different food sources: (a) paper pulp mill sludge, (b) domestic refuse, (c) cow manure and (d) rabbit manure. Values are means and standard errors

Table 1. Percentage of clitellate individuals of *E. andrei* and *E. fetida* fed on cow manure and rabbit manure (Ea = *E. andrei*, Ef = *E. fetida*)

	Cow manure		Rabbit manure	
Days	Ea	Ef	Ea	Ef
24	12	23	8	0
32	90	70	38	0
40	95	85	100	33
48	95	90	100	50
56	95	90	100	90
64	100	90	100	90
72	90	70	100	90
80	90	50	100	75
88	90	15	100	60
96	90	10	90	30
104	85	0	90	0

In the case of the domestic refuse (Fig. 1b), the fermentation process which occurred during the first 30 days caused the death of all *E. fetida* individuals. In contrast *E. andrei* overcame this critical period with low mortalities and slowly increased its biomass (4.86 mg/worm/day and maximum mean weight of 0.72 g).

The biomass values obtained for cow manure (Fig. 1c) were similar for both species during the first 60 days, and then *E. fetida* reached significantly higher weight ($p < 0.05$) until day 80 ($p < 0.01$). Despite *E. andrei* showing a higher growth rate (12.25 mg/worm/day and 0.43 g maximum mean weight) than *E. fetida* (8.80 mg/worm/day and 0.44 g maximum mean weight) weight losses were faster in the former species.

In rabbit manure (Fig. 1d), *E. andrei* rapidly increased its biomass reaching mean weights significantly higher than *E. fetida* between days 28 and 88 ($p < 0.01$). The mean growth rate for *E. andrei* was 12.78 mg/worm/day and the maximum mean weight was 0.77 g, whereas values of 8.06 mg/worm/day and 0.59 g respectively were recorded for *E. fetida*.

A stabilization and, later, weight loss was observed in cow and rabbit manures after the initial biomass increment, possibly due to nutritional exhaustion.

With regard to clitellum development, the first clitellate individuals of both species appeared on day 24 in cow manure, but after day 32 the number of mature *E. andrei* was higher than for *E. fetida* (Table 1) and this difference was maintained during the whole experiment because in *E. fetida* the clitellum regressed after day 64, so at the end of the experimental period only mature specimens of *E. andrei* were present. When rabbit manure was tested, the results were similar (Table 1). Again *E. andrei* acquired the clitellum first (the first clitellate individuals appeared on day 24 and on day 40 all individuals had the clitellum) whereas *E. fetida* started to develop this structure after 40 days, reaching the maximum maturation percentage between days 56 and 72. Thereafter clitellum regression took place so after day 96 no clitellate *E. fetida* specimens were present.

In relation to cocoon production, when the nutritional source was domestic refuse *E. andrei* was the only species depositing cocoons (1.47 cocoons/clitellate worm/week); in the case of cow manure the production rate was 1.47 cocoons/clitellate worm/week for *E. andrei* and 1.33 for *E. fetida*. The values obtained for rabbit manure were 2.17 cocoons/clitellate worm/week and 1.26 cocoons/clitellate worm/week, respectively.

Pure and mixed cultures of E. fetida and E. andrei

The initial individual numbers in pure cultures were 124 for *E. andrei* and 107 for *E. fetida* and in the mixed population 49 *E. andrei* (Ea*) and 68 *E. fetida* (Ef*). Mortalities were always low, both in pure cultures (*E. andrei* 13.2%, *E. fetida* 6.7%) and mixed ones (Ea* 10.4%, Ef* 4.4%).

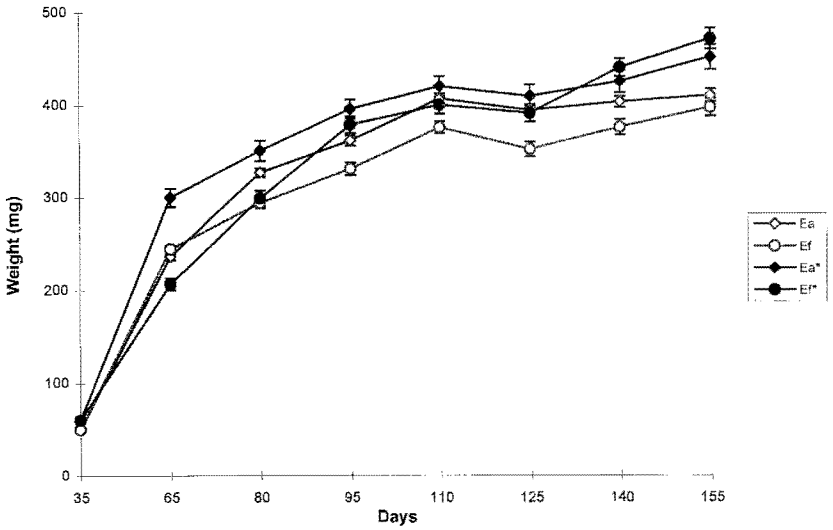

Fig. 2. Growth of *E. andrei* and *E. fetida* in pure (Ea and Ef) and mixed cultures (Ea* and Ef*). Values are means and standard errors

When the weight increments are compared (Fig. 2), it can be seen that the pattern of growth in the three cultures was similar. In the pure cultures, and during the first 110 days, a gradual and continuous biomass increment was recorded (Ea 4.67 mg/worm/day, Ef 4.40 mg/worm/day); then *E. andrei* significantly increased its weight ($p < 0.01$) until day 140, and then the growth rates of both species became similar (Ea 3.01 mg/worm/day, Ef 2.92 mg/worm/day). In the mixed culture, growth rates were similar for both species, although *E. andrei* (Ea*) gained weight more rapidly than *E. fetida* (Ef*) during the first 80 days ($p < 0.01$).

When pure cultures are compared to mixed ones, it can be seen that *E. andrei* showed a higher growth rate in the mixture than in isolation ($p < 0.05$) but after day 110 there are no significant differences. With regard to *E. fetida*, weight increments were greater in pure cultures in the initial stage but after day 80 the situation was reversed and the growth rate was significantly higher in the mixture than in pure cultures ($p < 0.01$).

E. andrei reached the mature stage before *E. fetida*, both in pure and mixed populations. Seventy days were necessary for 50% of individuals of *E. andrei* to have a well-developed clitellum whereas *E. fetida* needed 80 days, *E. andrei** 65 days and *E. fetida** 85 days. Later, the precentage of clitellate worms became similar in all the populations tesed, around 95%.

There were no significant differences in cocoon production and the mean rates were 2.34 cocoons/clitellate worm/week for *E. fetida* and 2.14 for *E. andrei*.

Morphological characterization of cocoons and hatchlings

A biometric study of cocoons from both species was carried out and the results are shown in Table 2. The cocoons from *E. fetida* were significantly larger and more spherical than the ones from *E. andrei* ($p < 0.01$), although no significant differences were detected in relation to their length. A few cocoons from *E. andrei* (14.43%) showed atypical morphologies (constrictions in the middle, one end abnormally long or with an irregular shape), which were not detected for *E. fetida*. These atypical cocoons seemed to be associated with the presence of deformed clitella in *E. andrei* and this was confirmed by culturing these abnormal specimens and obtaining irregular shaped cocoons from them.

Table 2. Biometric parameter of cocoons and hatchlings from *E. andrei* and *E. fetida*. Values as means ± S.E., N = 50. L = length, A = width, W = weight. The results of ANOVA and Fisher's LSD test are shown by asterisks (*$p < 0.01$)

	E. andrei	*E. fetida*
Cocoons:		
L (mm)	4.50 (0.10)	4.62 (0.08)
A (mm)	2.59 (0.02)	2.85 (0.03)*
L × A	11.56 (0.21)	13.44 (0.33)*
L/A	1.72 (0.04)	1.64 (0.05)
Hatchlings:		
L (mm)	9.86 (0.23)	10.03 (0.41)
A (mm)	0.68 (0.02)	0.76 (0.02)
W (mg)	3.62 (0.26)	3.08 (0.35)

E. fetida laid cocoons close to the surface, so the majority of them (81%, n = 368) were found in the first 4 cm and only 45% of the cocoons (n = 237) were recorded for *E. andrei* in the same layer.

The viability of the cocoons was high for both species, both in pure and mixed cultures (Ea: 88.1%, Ef: 88.3% and 88.2% in the mixture). The hatching time was also similar and ranged between 14 and 24 days for *E. fetida* (26.6 ± 0.340) and between 12 and 39 days for *E. andrei* (25.3 ± 0.397), these differences being significant ($p < 0.05$). *E. fetida* produced an average of 3.75 ± 0.294 newly-hatched individuals, significantly higher ($p < 0.05$) than for *E. andrei* (3.06 ± 0.238 hatchlings/cocoon). No significant differences were detected with regard to the biomass per cocoon for *E. andrei* and *E. fetida* (11.02 ± 0.610 mg and 10.54 ± 0.521 mg, respectively), or for the hatchlings, for any of the biometric parameters measured (Table 2).

F1 from the mixed population

Results showed a clear dominance of *E. andrei* during the first 80 days (Ea* = 373 individuals or 81%, Ef* = 88 individuals or 19%). After 95 days the differences were reduced (Ea* = 535 individuals or 60%, Ef* = 358 individuals or 40%) and after 110 days reversed (Ea* = 519 individuals or 42%, Ef* = 703 individuals or 57%). This could be explained by the fact that *E. andrei* reached maturity more rapidly and because of the higher number of hatchlings per cocoon in *E. fetida*. With regard to biomass, *E. andrei* showed a clear dominance during the whole experimental period due to its higher growth rate (84%, 75% and 59% for *E. andrei* after 80, 95 and 110 days, and 16%, 25% and 41% for *E. fetida*, respectively).

Discussion

The population dynamics of both species play an important role as a distinguising criterion, although their responses to the different food sources was similar with the exception of the domestic refuse. In general, growth rates and cocoon production were higher in *E. andrei*. These results are in agreement with those obtained by Haimi (1990), who recorded higher growth rates and cocoon production in *E. andrei* (10.76 mg/worm/day and 0.44 cocoons/worm/day) than *E. fetida* (9.25 mg/worm/day and 0.26 cocoons/worm/day) when they were fed on oat flakes. Reinecke & Viljoen (1991a), found a higher cocoon production for *E. andrei* but Sheppard (1988) reported similar production for both species.

Our results showed no significant differences between pure and mixed cultures and no negative effects on mortality, growth patterns, cocoon production and cocoon viability,

although Abbot (1980) and Rouelle et al. (1987) pointed out that the presence of *E. fetida* as well as *E. andrei* could alter growth and survival of other species due to either a better assimilation efficiency or excretion of toxic sustances. The differences in the growth rates recorded here are better explained as a different reproductive strategy than as a negative interaction; *E. andrei* grew and reached sexual maturity more rapidly than *E. fetida*, producing cocoons sooner and thus becoming dominant in the following generation. Sheppard (1988), reported a lower hatching rate for the cocoons produced in mixed cultures of *E. andrei* and *E. fetida*. This contrasts with our results with no significant differences between pure and mixed cultures detected.

When the results obtained from the pure cultures are compared with those of the earlier experiment, a disagreement in the time required for acquiring the clitellum was observed, less time was necessary for the eight specimens of *E. fetida* and *E. andrei* to have a well developed clitellum (see Table 1). This could be explained in terms of competition, the pure cultures in the second experiment supported a greater initial number of individuals (124 *E. andrei* and 107 *E. fetida*) and this probably had an effect on maturation.

Despite the similar appearance of the cocoons of both species, the cocoons of *E. fetida* were larger than those of *E. andrei*; and only *E. andrei* produced abnormal cocoons in direct relationship with deformed clitella, a finding in agreement with Terhivuo & Valovitra (1974). Our results are also consistent with those of Haimi (1990), who observed that the fresh weight of the cocoons of *E. fetida* (21.8 mg per cocoon) was greater than those of *E. andrei* (18.1 mg per cocoon). In relation to vertical distribution, Reinecke & Viljoen (1991 b) recorded a more superficial deposition for *E. fetida*, finding that 84% of the cocoons were laid in the top 6 cm.

The incubation period was slightly longer for *E. fetida*, which is consistent with the results obtained by Venter & Reinecke (1988), ranging between 14 and 44 days ($x = 23$ days). In addition, the number of individuals emerged per cocoon was also greater for *E. fetida* (3.75 individuals per cocoon) than *E. andrei* (3.06 individuals per cocoon) which falls within the intervals given by Sheppard (1988) (*E. andrei* = 2.86 and *E. fetida* = 4.55) and by Haimi (1990) (*E. andrei* = 2.2 and *E. fetida* = 3.4) and is in contrast to those obtained by Reinecke & Viljoen (1991a), who recorded a higher number for *E. andrei* (*E. andrei* = 3.31 and *E. fetida* = 2.33).

In conclusion, although growth rates were similar in both species, some differences with regard to their reproductive strategy were detected. Thus, *E. fetida* showed a higher cocoon production and more hatchlings per cocoon than *E. andrei* and then, when both were reared together, a clear dominance of the former species is anticipated. But, *E. andrei* requires less time to reach sexual maturity than *E. fetida* because of its rapid intitial growth and it is then able to start cocoon production sooner and this represents a competitive advantage in the following generation. The results obtained here can be interpreted in the context of the r and k continuum of life history strategies and, according to this, *E. andrei* seems to be a more extreme r strategist than *E. fetida* as evidenced by more rapid growth and reproduction.

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