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Mutualism between earthworms and soil microflora

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Summary. The gut mucus production of different earthworms species has been measured in order to verify the mutualistic hypothesis between earthworms and soil microflora. Earthworms of different origin (tropical and temperate) and ecological category (endogeic, anecic and epigeic), individuals of the same species occuping different soils, and earthworms of different species from the same soil, have been studied. Intestinal mucus production suggests the existence of the mutualistic digestion system. The mucus percentage in the gut was greater in the epigeic than in both anecic and endogeic. Nevertheless, the relative enrichment was higher in the endogeic species. The organic matter content of the substratum seems to play a regulating role in the mutualistic system, because the greater the substratum organic matter content the lower the relative enrichment. Generally, in temperate zones more mucus was added to the substratum, suggesting that the low temperature do necessary a greater microflora stimulation.

Key words: Earthworms, mutualism, mucus, tropical species, temperate species

Introduction

Many soil invertebrates and among them the earthworms have developed interactions with soil microorganisms to exploit soil organic matter (Lavelle 1986).

Geophagous endogeic earthworms have a poor digestive enzymatic capacity (Lavelle 1983), whilst the edaphic microflora have the capacity to degrade almost any kind of organic substratum. This fact has lead to the postulation of a mutualistic relationship between soil microflora and earthworms to promote the digestion of organic compounds.

Soil microorganisms find suitable conditions in the gut of earthworms for their activity: high water content, neutral pH and high concentrations of intestinal mucus, which is an easily metabolizable organic matter resource (Barois & Lavelle 1986; Barois 1992; Trigo & Lavelle 1993). This mucus is a mixture of low molecular weight hydrosoluble compounds (Martin et al. 1987), and its effect has been called a "priming effect" by Jenkinson (1966).

Intestinal mucus production represents a high energetic cost for earthworms. They produce large quantities in the foregut (Martin et al. 1987; Trigo & Lavelle 1993, 1995), but it is reabsorbed during the gut transit (Lavelle et al. 1983a), mainly in the hindgut, so the micro-

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organisms gradually recover their initial metabolic status in both the medium and the posterior gut.

The mutualistic digestion system seems to be clear in endogeic earthworms that feed on very stabilized and humified organic matter. Zhang et al. (1993); Lattaud et al. (1997) and Garvín (com. pers.) have demonstrated that cellulase and mannase found in the gut of *Pontoscolex corethrurus* and *Hormogaster elisae*, respectively, are produced by the ingested microflora because they do not appear in *in vitro* intestinal tissue cultures.

In order to confirm the mutualistic system, intestinal mucus production has been compared among species from different ecological categories, from temperate and tropical regions and of native or exotic origin. Mucus production also has been measured in individuals of the same species but inhabiting soils with different organic matter content or of different species found in the same soil.

Materials and Methods

The study was carried out with 6 tropical and 4 temperate species from Mexico and Spain, respectively (Table 1).

Table 1. Studied species and organic matter content (% C) from the substrata

Species	n	Ecological category	Origin	Site	Substratum	% C
Eisenia andrei	8	Epigeic	*Tropical*	Xalapa (Mexico) Exotic	Coffee pulp	55.7
Eisenia andrei	11	Epigeic	*Temperate*	Vigo (Spain) Native	Cow manure	10.86
Perionyx excavatus	5	Epigeic	*Tropical*	Xalapa (Mexico) Exotic	Coffee pulp	55.7
Octodrilus complanatus	16	Anecic	*Temperate*	Madrid (Spain) Native	Forest soil	7.06
Balanteodrilus sp.	5	Endogeic	*Tropical*	Xalapa (Mexico) Native	Inceptisol Savanna soil	1.10
Hormogaster elisae	14	Endogeic	*Temperate*	Madrid (Spain) Native	Subneutrophilic pasture soil	0.97
Hormogaster elisae	6	Endogeic	*Temperate*	Madrid (Spain) Native	Gall oak soil wood *Quercus faginea*	2.01
Glossocolecidae sp.	5	Endogeic	*Tropical*	Xalapa (Mexico) Native	Inceptisol Savanna soil	1.10
Octolasion cyaneum	10	Endogeic	*Temperate*	Madrid (Spain) Native	Orchard soil	3.56
Pontoscolex corethrurus	4	Endogeic	*Tropical*	Xalapa (Mexico) Native	Inceptisol Savanna soil	1.10
Pontoscolex elongata	9	Endogeic	*Tropical*	La Mancha (Mexico) Exotic	Alfisol	1.13

The earthworms were kept in the substratum where they were taken, under laboratory conditions. Epigeic species from tropical regions were found in coffee pulp, *Pontoscolex excavatus* and *Eisenia andrei*, were also found in temperate regions in cow manure.

The earthworms were killed by immersion in boiling water for some seconds. Then, each earthworm was weighed and divided into 3 equivalent parts, anterior (A), medium (M) and posterior (P), between the gizzard and the pygidium. Each part was dissected longitudinally. The dorsal vessel was removed and the coelomic fluid was dried with a filter paper. Then, the gut wall was opened and the intestinal content was removed with a spatula. For mucus extraction, the intestinal content was diluted in 25 ml of water at 60 °C. The mixture was shaken for 3 minutes and filtered through a Millipore membrane (0.20 µm). The filtrate was frozen and lyophilized. The substratum retained on the filter was dried at 105 °C. Both the filtrate and the substratum were weighed; their addition represents the weight of the gut content and their respective proportions are the mucus and the organomineral part of the gut content. The same procedure was carried out on both the culture substrata and fresh casts.

The results were statistically analysed by one way ANOVA tests. The mucus content of species from different ecological categories and origins was compared, as well as the mucus content of the gut of individual species with that of its respective casts and substratum.

Results

All the studied species showed great mucus production in the anterior part, with concentrations 10 to 176 times that found in the substrata. These concentrations are rapidly reabsorbed during the gut transit, so the ratio between anterior and posterior gut mucus content varied from 1.46 to 7.91. The mucus content in casts was very low, although much higher than the corresponding substratum.

The mucus percentages in the gut contents of the species from different ecological categories are shown in Fig. 1. The epigeic species had higher intestinal mucus production than endogeic ones. However, when the quantity of mucus of the anterior part is related to that in the substratum [relative enrichment, i.e. mucus in the anterior gut / mucus in the substratum (Fig. 2)], it is apparent that the endogeic earthworms added relatively more intestinal mucus than epigeic species.

In the tropics both epigeic species *E. andrei* and *P. excavatus* showed 8–11 times more mucus in the anterior gut than in the substrata. In the temperate *E. andrei* the ratio was 23. For anecic species only the temperate *Octodrilus complanatus* was studied. This species had 17 times more mucus in the anterior gut than in the substratum. In the case of endogeic worms, the concentration of mucus in the anterior gut was 35–90 and 18–24 times that in the substrata for the temperate *H. elisae*, from 2 different soils, and *Octolasium cyaneum* and the tropical *Pheretima elongata*, *P. corethrurus*, *Gen. Nov.* (Glossoscolecidae) and *Balanteodrilus sp.*, respectively. So, it seems that both epigeic and endogeic temperate earthworms produce more mucus than do the tropical species.

The mucus produced in the anterior gut varied with the organic matter content of the substratum, as is illustrated in Table 2 for the mucus of both *E. andrei* from two different substrata and *H. elisae* in two soils. It can be seen that the higher is the substratum organic matter content the higher is the percentage of mucus produced. Considering the relative enrichment, mucus production is greatest in the poorest organic matter substrata.

Intestinal mucus production also showed specific variability, as deduced by comparing the quantity of mucus produced by different species inhabiting the same soil: *P. corethrurus*, found in a savanna soil, had a selective enrichment varying from 91 to 177. In Mexico the

Table 2. Organic carbon of substrata (%C); % mucus in the anterior part of the gut (%A), % mucus in the substrata (%S) and A/S

SPECIES	%C	% A	% S	A/S
Eisenia andrei – tropical – coffee pulp	55.7	78.83	7.06	11.17
Eisenia andrei – temperate – cow manure	10.9	36.44	1.57	23.21
Hormogaster elisae – temperate – subneutrophilic pasture	1.0	4.95	0.08	61.87
Hormogaster elisae – temperate – wood *Quercus faginea*	2.0	6.38	0.18	35.45

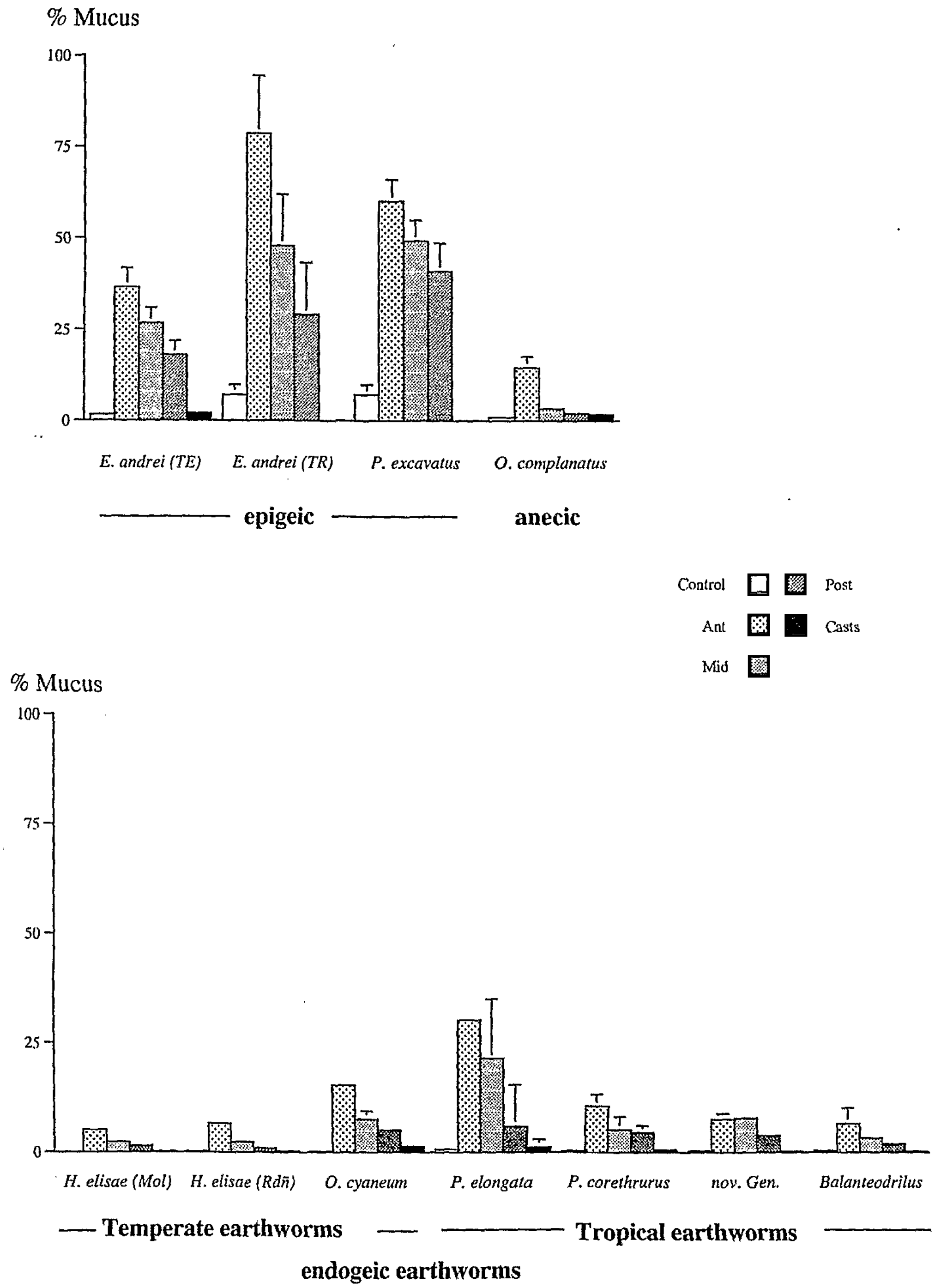

Fig. 1. % Mucus in substrata, anterior, medium and posterior parts of the gut and casts. TE: Temperate, TR: Tropical, Mol: El Molar, Rd: Redueña

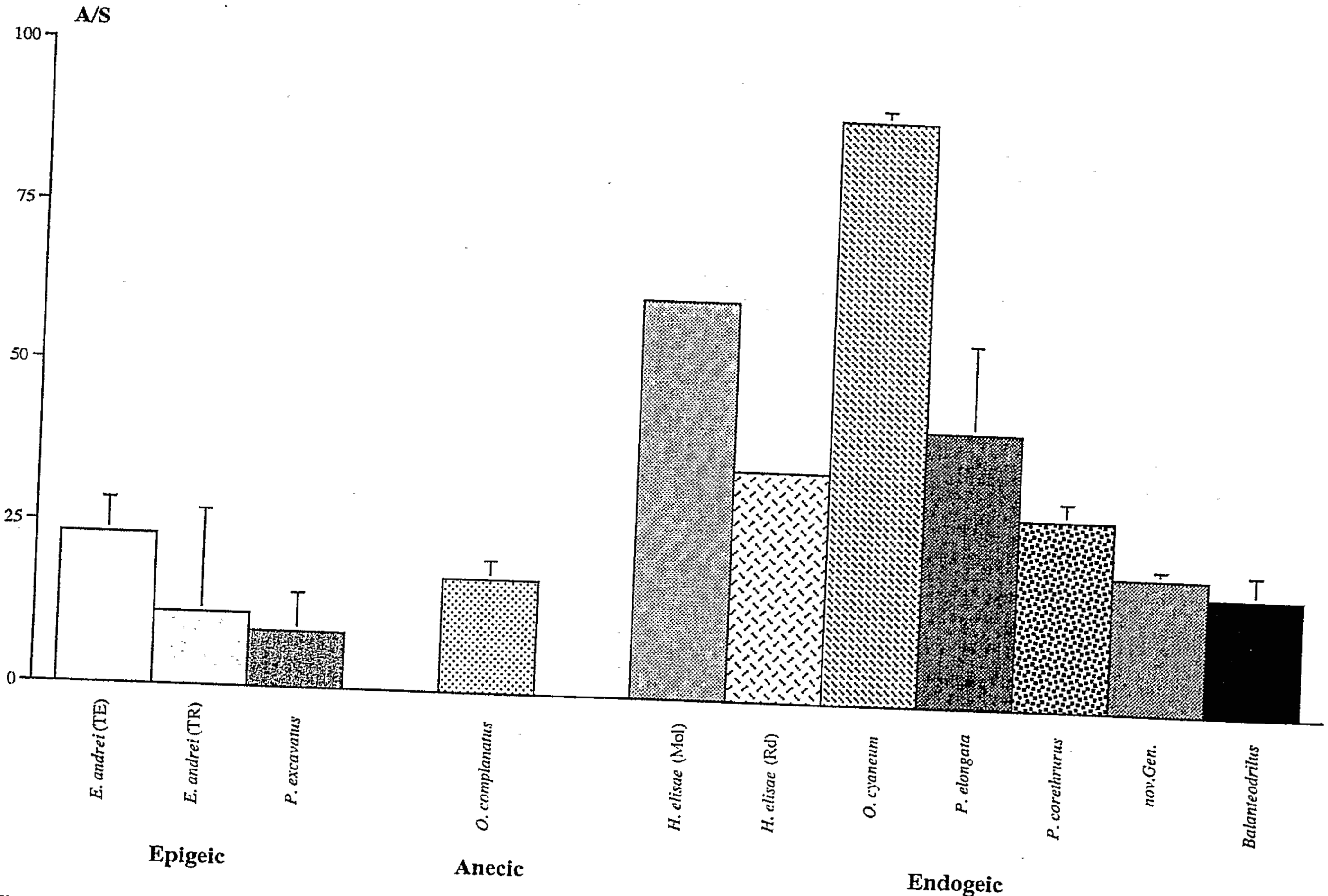

Fig. 2. Selective enrichment in the different ecological categories. TE: temperate, TR: tropical, Mol: El Molar, Rd: Redueña. A/S: mucus in the anterior gut/mucus in the substratum

Table 3. ANOVA test for the total mucus production among earthworms grouped in ecological categories and origin of the studied species (*TE*: Temperate; *TR*: Tropical). * 95 % significance; ** 99 % significance

	Epigeic (*TE*)	Epigeic (*TR*)	Anecic	Endogeic (*TE*)	Endogeic (*TR*)	Casts	Substrata
Epigeic (*TE*)	–	**	**	**	**	**	**
Epigeic (*TR*)		–	**	**	**		**
Anecic			–	*	**	**	**
Endogeic (*TE*)				–	–	**	**
Endogeic (*TR*)					–	*	–
Casts						–	–

exotic species *P. elongata* and *P. corethrurus* had a higher mucus production than the native species *Nov. gen.* (Glossoscolecidae) and *Balanteodrilus*.

Table 3 gives the results of the ANOVA of the total intestinal mucus production of the different species, their ecological categories, their substrata and their casts. Significant differences of mucus production were observed between the ecological categories. Comparing species of tropical or temperate origin, there were significant differences only between the epigeic earthworms. Tropical endogeic species produced less mucus than temperate species but the differences were not significant. The mucus content in the gut was always significantly higher than in their respective substratum and casts, independently of both the origin and the ecological category.

Discussion

The mutualistic soil organic matter digestion system was postulated by Lavelle (1986). Different authors have studied it in several tropical species (Lavelle et al. 1983b; Barois & Lavelle 1986; Barois 1992; Martin et al. 1987) and in two temperate species (Trigo & Lavelle 1993, 1995); thus a global vision of this process exists.

The functioning of the mutualistic digestion system in the different ecological categories is little known because it only has been studied on endogeic species, except for *Amynthas corticis* and *Amynthas gracilis* (Barois 1992), two non typical epigeic earthworms which are preferably geophagous, and thus should be recognised as epiendogeic (Bouché 1972). The results of this paper show that temperate and tropical endogeic species add more mucus to the soil than either anecic or epigeic species. These latter feed on substrata that are rich in organic matter and/or whose organic matter is easily assimilable, therefore they probably do not need to induce an important stimulation of the soil microflora. Thus the species that had a relatively less enrichment in mucus were the epigeics *P. excavatus* and *E. andrei* and the anecic *O. complanatus*. Judas (1992) observed that *O. cyaneum* ingests mainly soil with few organic particles, so it is a true endogeic species and its enrichment in intestinal mucus was high as expected, as were also the other endogeic species compared to the epigeic and anecic species. Endogeics have a more intense relationship with the ingested microflora and therefore they need to rapidly and greatly stimulate it with high mucus production.

Barois & Martin (1989) concluded that the earthworm-microflora interaction model in all endogeic species is basically the same although they have different ecology and feed in soils of different energetic richness; however there are quantitative differences depending on other factors. The organic matter content of the ingested soil seems to play a regulating role in the mutualistic system. The results obtained with *H. elisae* from two sites (El Molar and Redueña) suggest that the greater the amount of organic matter in the substratum the greater the amount of mucus that is added in the anterior part of the gut, but the lower the percentage of selective enrichment (A/S = 35.45 in Redueña and 61.87 in El Molar). Although the differences between our sites were not significant, this fact was probably attributable to the different number of samples. The same results were observed in both *E. andrei* cultured in two different kinds of substrata and in *P. corethrurus* comparing our data with those of Barois & Lavelle (1986).

This relationship between the substratum organic matter content and the mucus added to the anterior part of the gut had not been noticed until now. However its existence might have been suspected from the data on mucus content and respiratory activity in both soil and casts of *Millsonia anomala* cultured in enriched soils (Lavelle et al. 1983b). These authors remarked that earthworms that feed in soil with low organic matter content produce casts with higher mucus concentration than the soil, which could be due to higher relative enrichment and/or soil selection.

Concerning differences between the temperate and tropical zones, generally the temperate endogeic species inject more mucus into the ingested soil than do tropical species, in relative terms. Similarly with the epigeic earthworms *E. andrei* from temperate regions, which produced more intestinal mucus than this species from tropical zones.

It has been suggested that the high temperature of the tropics increases the mutualistic digestion system efficiency, due to greater microbial activity (Barois & Lavelle 1986). The microbial respiration in *P. corethrurus* gut at 15 °C is half that at 22 °C and at 28 °C it is 6–10 times higher (Barois 1987). Lavelle et al. (1995) affirm that the mutualistic digestion system becomes more efficient with increasing temperatures. This could explain why in this study there was higher relative production of mucus in the temperate species. High temperatures increase microbial efficiency, and there is no need for the production of large amounts of intestinal mucus; knowing the energetic cost that this mucus production represents. In temperate zones with lower temperatures a higher amount of mucus could be needed to stimulate the microflora. Scheu (1991) has estimated that the temperate endogeic *O. lacteum* daily looses 0.5 % of its total C by intestinal mucus excretion.

For the tropical *E. andrei* the substratum (coffee pulp) had a higher organic matter content than the temperate substrate (cow manure), so both effects, i.e. temperature and organic matter quality, could be acting together. Thus it can be said that there is a conditional mutualism between the earthworms and the soil microorganisms having a "mutual exploitation for mutual gain" (Bronstein 1994). The parameters that condition the intensity of the relationship are the earthworm digestive enzymes, the soil organic matter (quantity and quality) and the temperature. The exotic species seem to have the strongest relationship with the microflora which increases their fitness capacity.

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