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Original article

Selective feeding of the earthworm *Hormogaster elisae* (Oligochaeta, Hormogastridae) in laboratory culture

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Abstract

Selective feeding of the earthworm *Hormogaster elisae* was investigated by granulometric analysis, chemical fractionation and physical fractionation of the soil in which representatives were cultivated, and of their casts. *H. elisae* behaved as an endogeic species, mainly consuming soil from which it weakly but positively selected the organic fractions of greatest size (mainly free organic matter) and negatively selected the largest mineral fraction (coarse sand). No significant selection of intermediate size fractions was recorded. The 0–2 μm granulometric fraction (clays) was also selected; the results suggest that some of the organic components of this size fraction can also be used by the species.

Keywords: Earthworms; Selective feeding; Soil organic matter

1. Introduction

Earthworms can use a wide variety of organic materials for food [9], but not all earthworm species' feeding mechanisms are well known. Several authors have divided earthworms into groups according to their ecological characteristics, including their feeding habits. Litter, topsoil and subsoil feeders [17], or epigeic, anecic and endogeic species [2], have been described. The endogeic species are divided into polyhumic, mesohumic and oligohumic species [15]. The epigeic and anecic species often behave as litter-feeders, while the endogeic species are more geophagous, consuming large quantities of soil in order to meet their organic matter needs. Piearce (1978) describe *Lumbricus castaneus* and *Lumbricus rubellus* mainly as litter-feeder, whereas *Aporrectodea caliginosa* and *Allolobophora chlorotica* consume well-decomposed organic detritus. *A. longa* and *Dendrobaena mammalis* have more intermediate dietary requirements [25].

Other authors, however, maintain different views [20–22] and indicate that earthworms, including the endogeic species, consume relatively fresh matter, sometimes accompanied by certain quantities of microbial biomass.

In any event, the endogeic species have to consume large quantities of soil if they are to cover their energy requirements; their diet is based on more or less decomposed organic detritus which is energetically poor and sometimes difficult to digest.

An interesting question regarding the feeding of these species concerns whether they ingest 'bulk soil',

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or select among soil a fraction richer in organic matter. The latter types of behaviour would provide a richer diet and reduce the effort needed to cover energy requirements.

Lavelle and Spain (2001) [16] state that most endogeic earthworms may select organic and mineral soil fraction. Up to 15% differences in the plant debris content of the diets of some species are known [3]. In many species the organic matter content of the casts is greater than that of the soil, further suggesting that food selection is practised [8]. With respect to the mineral fraction, it is well known that the casts of some species have a less abundant coarse fraction and larger fine fraction than the original soil from which they were produced [9]. The casts of *A. molleri* have 56.40% more clay and 48.72% less coarse sand than the original soil [30]. The casts of *Millsonia anomala*, *Pontoscolex corethrurus* and *Dichogaster terrae-nigrae* usually have more fine material and less coarse material than the original soil. However, in a few cases, sand particle selection in clay-rich soils occurs [11,19]. This would appear to indicate that some active selection is made of what is ingested. An alternative explanation is the possible breakage of the larger mineral particles ingested by the action of the gizzard, although this is rejected by Lee [18] who indicates that the pressure exerted by this organ is too small to break up mineral particles.

Knowledge of earthworm feeding and food selection processes is necessary if we are to understand the true role of these organisms in the soil and their influence on organic matter cycles. The species examined in the present study was *Hormogaster elisae*, an endogeic earthworm endemic to the centre of the Iberian Peninsula which lives on sandy soils poor in organic matter [14]. *H. elisae* is able to make vertical movements in the soil over the year depending on the soil moisture condition [31].

Several studies have shown that earthworms influence the dynamics of soil organic matter [12,18,21]. They increase the rate of nutrient recycling, particularly that of nitrogen [13], and therefore have an important impact on soil fertility. The organic matter of the soil is a complex mixture of different substances ranging from relatively non-degraded free organic matter to humic substances such as fulvic acids (FA), humic acids (HA) and humins. Humic substances make up a relatively stable proportion of the soil carbon content, regulate its cycling and the liberation of other nutrients (nitrogen, phosphorus and sulphur), have an impact on the potential fertility of the soil, favour the growth of plants (e.g. they promote the acquisition of Fe), retain water, and provide thermal insulation [23].

The relative importance of the different soil organic fractions in earthworm diets is, however, poorly understood. Some authors indicate that, irrespective of their ecology, earthworms feed on recently deposited, easily decomposable organic matter, and suggest that this consists mainly of soil organic particles > 50 µm as well as some microbial biomass [22]. Although soil-feeding earthworms ingest large quantities of humic substances with the soil, these are generally not assimilated.

Given this background, the aim of this study was to assess the possible selection of food by *H. elisae* in laboratory culture. Analyses were performed to determine whether organic material is selected at the chemical level, to see what types of organic and mineral particles are selected, and therefore, to characterise what this species ingests.

2. Materials and methods

Soil and earthworms were collected by digging and manual separation at El Molar (Province of Madrid, Spain: U.T.M. 30TVL5210). All earthworms were maintained in the laboratory in the same soil in which they were captured.

2.1. Granulometric study of the soil with destruction of the organic matter

Three types of microcosm were prepared, each containing 340 g of dry soil of different texture (soil sieved to 4, 2 or 0.6 mm). Twenty grams of each (control soils) were removed for granulometric analysis; the remaining soil was brought to a moisture level of 20%. Eight replicates of each texture treatment were established, making a total of 24 microcosms.

A weighed earthworm was introduced into each microcosm, where it was left for 10 days at 18 °C. Surface casts were removed daily (all adhered soil particles were removed with a small brush) and frozen until analysis. At the end of the 10-day period the worms were weighed again and all deep-layer casts removed [8]. After destruction of the organic matter by the addition of H_2O_2, a granulometric analysis of 20 g of control soil and of the casts in all the soil texture treatments was performed using the Robinson pipette method [26]. The percentages of total sands [coarse sand 200–2000 µm plus fine sand 50–200 µm], total loam [coarse loam 20–50 µm plus fine loam 2–20 µm] and clay [< 2 µm clay] were determined in each.

2.2. Physical and chemical fractionation

Ten experimental and two control microcosms with 320 g of soil (moisture level 20%) were set up. Five weighed earthworms were placed in each experimental microcosm and cultivated for 7 days at 18 °C. Surface casts were collected daily, removing any adhered soil particles with a small brush, and frozen until analysis. At the end of the 7-day period the worms were weighed again and the non-ingested soil and deep-layer casts separated [8].

The casts and non-ingested soil from the 10 experimental microcosms were mixed to homogenise the sample and dried at room temperature. The two control soils underwent the same procedure. Samples were then taken from each and subjected to chemical fractionation (six replicates) and physical fractionation (two replicates).

2.2.1. Physical fractionation with no destruction of the organic material

This was performed on 40 g samples using the method of Feller [10] with some modifications. This method is based on the idea that the mineral fraction is heavier than the organic fraction, and that they can therefore be separated by suspension in distilled water. This provided mineral and organic fractions of 250–2000, 50–250 and 20–50 µm, and organo-mineral fractions of 2–20 and 0–2 µm.

The soil at El Molar is developed from granites and gneisses, which means it has a certain amount of free mica. Owing to its low weight and laminar structure, some remains in suspension with the organic fractions. To calculate the error caused by this, small quantities of each of the 20–2000 µm organic fractions were incinerated in a muffle furnace at 600 °C for 24 h, after which time only the mineral component (mainly mica) remained. Using the initial and incineration-corrected organic fraction data, a regression line was produced for calculating the true organic percentages of each fraction as well as their C and N content and C/N ratio.

The total carbon (%C) content of control soil and each of the size fractions was determined by the Anne method [26] adapted for a microplate reader. Total nitrogen (%N) was determined by the Kjedahl method [4]. The C/N ratio was then calculated for each.

In this type of 'textural' study made with destructured soil, the importance of the soil aggregates was not considered and perhaps in the future it would be suitable to integrate aggregate structure analysis together with the textural analysis. In the soil of El Molar, sand particles and coarse organic matter (litter fragments) are probably free, and loam, clay and humified organic matter are structured into aggregates. Soil 'structure' can be important in the food selection processes, because the elemental particles form aggregates that could have influence on the real selection of food, most of all the aggregates made of a mixing between quite highly humified organic matter, loam and clay minerals.

2.2.2. Chemical fractionation

This was performed using the method of Dabin [6] as modified by Almendros et al. [1]. Samples were treated with H_3PO_4, which extracts the non-humified organic matter (free organic matter plus a fraction of water soluble compounds known as free fulvic acids [FFA]) and eliminates the carbonates and bivalent cations from the exchange complex, favouring later extractions.

The sample was then treated with $Na_4P_2O_7$ to obtain the total humic substances (THS), from which the HA were separated using H_2SO_4 and NaOH as recommended by the International Humic Substance Society [5]. The FA content was calculated using the equation $FA = THS - HA$.

To determine the extractable humin, demineralising agents such as $Na_2S_2O_4$ and HF–HCl were used. The non-extractable humin was calculated as the difference between the %C of the sample and the sum of the free organic matter, FFA, TSH and extractable humin fractions [1].

2.3. Statistical analysis

The normal distribution of the variables was tested using the Shapiro–Wilks test. The results were analysed by one-way ANOVA and simple regression. Significance was set at 95%.

3. Results

The earthworms gained weight during the experiment, from 11.60 + 1.33 to 11.97 + 2.07 g in the fractionation microcosms, from 3.99 + 0.66 to 4.44 + 0.48 g (4 mm series), 4.08 + 0.72 to 5.04 + 0.66 g (2 mm series), and 3.86 + 0.75 to 4.09 + 0.80 g (0.6 mm series) in the soil selection microcosms, indicating they had been kept in good condition and had acclimatised to the experimental conditions.

3.1. Granulometric study

The casts of *H. elisae* produced in the three texture environments were different to the control soil in terms of every fraction. Table 1 shows the significant negative percentage variation in total sands (–7.28%, –4.58% and –2.14%) and the significant positive percentage variation in clays (57.06%, 27.21% and 17.58% for the 4, 2 and 0.6 mm textures, respectively). With respect to the remaining fractions, the species showed differences mainly in intensity depending on the soil texture in question. In the 4 and 2 mm soils, *H. elisae* showed a certain negative selection of the > 250 µm coarse fraction, and a certain positive selection of the remaining mineral particles, especially the clays. This selection was reduced when the soil texture was finer. In the 0.6 mm soil the percentage variations of all the fractions was negative, except for coarse sand (2.51%) and clays (17.58%). The differences in fine sands, total sands and clays between the casts and control soil were significant for all textures. In the 0.6 mm soil, the earthworms ingested proportionally more clay (17.58%) than that making up the composition of the soil. However, this was even more noticeable in the coarser textured soils (27.21% in the 2 mm soil and 57.07% in the 4 mm soil).

The selection of certain soil fractions was therefore significant, if not very strong, since in the 4 mm soil microcosms (that most like the natural EL Molar soil) the casts only had 6% less sand and a little more than 4% extra clay.

3.2. Physical fractionation

The method used to correct the organic fractions for mica contamination was very effective ($r = 0.998–0.966$, $P < 0.01$). Table 2 shows the corrected physical fractionation data. N could not be determined in some fractions since the samples were insufficiently large. Since only two replicates per sample were used, no statistical analysis was made.

The largest quantities of the 250–2000 µm mineral fraction were found in the non-ingested soil, followed by the control soil and finally the casts. The order of abundance of the 250–2000 µm organic fraction was control soil > casts > non-ingested soil. The 50–250 µm fraction was more abundant in the casts than in the control or non-ingested soil (casts > control soil > non-ingested soil). Similar results were seen for the organic compartment: casts > control soil > non-ingested soil.

The relativity high organic matter content of the casts (1.29%) and the lower values of the control soil (0.91%), and non-ingested soil (1.15%) indicate that part of this enrichment could be due to the fragmentation of larger particles.

Few variations were seen with respect to the 20–50 µm fraction. The mineral fraction was somewhat larger in the non-ingested soil (4.38%) than in the control soil (3.84%) and casts (3.15%).

The techniques used did not allow the separation of the organic and mineral 0–2 and 2–20 µm fractions, which limits the conclusions that can be drawn. The

Table 1
Granulometric study, percentage of each fraction and standard deviation (in brackets)

Texture		*N*	TS	TL	CS	FS	CL	FL	Clay
4 mm	Casts	6	76.58 a	11.52 a	62.86 a	13.72 a	2.04 a	9.48 a	11.89 a
			(1.11)	(0.50)	(0.81)	(0.42)	(0.46)	(0.48)	(1.55)
	Control soil	8	82.59 b	9.84 b	69.18 b	13.41 a	1.75 a	8.10 a	7.57 b
			(1.43)	(0.98)	(1.50)	(1.23)	(0.46)	(0.75)	(0.57)
	PM		–7.28	17.07	–9.14	2.31	16.57	17.04	57.07
2 mm	Casts	8	76.71 a	12.21 a	62.54 a	14.17 a	2.35 a	9.86 a	11.08 a
			(2.10)	(0.78)	(2.01)	(1.11)	(0.53)	(0.45)	(1.50)
	Control soil	8	80.39 b	10.89 b	67.42 b	13.01a	2.07 a	8.83 b	8.71 b
			(0.73)	(0.61)	(1.47)	(1.30)	(0.28)	(0.51)	(0.44)
	PM		–4.58	12.12	–7.24	8.92	13.53	11.66	27.21
0.6 mm	Casts	8	71.17 a	14.93 a	52.32 a	18.85 a	2.56 a	12.36 a	13.91 a
			(0.81)	(0.71)	(1.53)	(0.96)	(0.62)	(0.65)	(0.76)
	Control soil	8	72.73 b	15.44 a	51.04 a	21.69 b	2.92 a	12.52 a	11.83 b
			(0.88)	(0.95)	(1.79)	(1.27)	(0.74)	(0.48)	(0.71)
	PM		–2.15	–3.30	2.51	–13.09	–12.33	–1.28	17.58

Abbreviations: total sand (TS); total loams (TL); clays (Clay); coarse sand (CS), fine sand (FS); coarse loams (CL); fine loams (FL); PM: percentage variation in casts with respect to the control soil (percentage in casts – percentage in control soil/percentage in control soil) × 100. Lower case letters in the same column indicate significant differences (ANOVA, $P < 0.05$). *N*: number of replicates.

Table 2
Physical fractionation

	250–2000 μm		50–250 μm		20–50 μm		2–20 μm	0–2 μm
	MF	OF	MF	OF	MF	OF	MOF	MOF
CS								
GC%	57.11	0.40	15.76	0.91	3.84	0.19	8.28	13.31
C (%)	0.05	49.72	0.10	10.57	0.44	34.28	1.43	0.92
N (%)	0.004		0.005	1.62			0.18	0.14
C/N	12.68		20.88	6.53			7.97	6.60
NIS								
GC%	59.32	0.24	14.31	1.15	4.38	0.13	8.93	11.44
C (%)	0.05	49.06	0.10	9.79	0.48	23.50	1.40	0.92
N (%)	0.005		0.005	1.52			0.17	0.13
C/N	10.44		20.71	6.42			8.16	7.03
CASTS								
GC%	54.90	0.37	18.91	1.29	3.15	0.18	8.51	12.33
C (%)	0.05	53.84	0.12	10.58	0.38	32.06	1.16	0.86
N (%)	0.006		0.007	1.67			0.17	0.14
C/N	8.13		17.29	6.32			6.73	6.28

Values for the organic fraction (OF), mineral fraction (MF) and organo-mineral fraction (MOF), corrected using regression curves. GC%: granulometric composition (percentage). CS: control soil, NIS: non-ingested soil.

values for control soil, casts and non-ingested soil with respect to the 2–20 μm fraction were very similar (8.28%, 8.51% and 8.93%, respectively).

Table 3
1: Distribution of organic C in different fractions (% C). 2: Percentage of C in each fraction with respect to total soil C

		CS	NIS	CASTS
FOM	1	0.122 a	0.118 a	0.146 b
	2	20.85	20.85	23.21
FFA	1	0.020 a	0.016 a	0.023 a
	2	3.42	2.83	3.66
FOM + FFA	*1*	*0.141 a*	*0.133 a*	*0.169 b*
	2	*24.10*	*23.50*	*26.87*
HA	1	0.077 a	0.063 a	0.087 a
	2	13.16	11.13	13.83
FA	1	0.074 a	0.119 b	0.060 a
	2	12.65	24.56	9.54
THS	*1*	*0.150 a*	*0.201 b*	*0.147 a*
	2	*25.64*	*35.51*	*23.37*
HA/FA		*1.035 a*	*0.450 b*	*1.462 a*
EH	1	0.047 a	0.043 a	0.041 a
	2	8.03	4.06	2.70
NEH	1	0.247 ab	0.209 a	0.297 b
	2	42.22	36.93	47.22
%C		0.585 a	0.568 a	0.654 b
%N		0.115 a	0.101 a	0.141 b
C/N		5.087 a	5.604 a	4.461 b

FOM: free organic matter; FFA: free fulvic acids; THS: total humic substances; HA: humic acids; FA: fulvic acids; EH: extractable humin; NEH: non-extractable humin. Total organic carbon (%C), nitrogen (%N) and C/N ratio of the control soil (CS), non-ingested soil (NIS) and casts (CASTS). Small case letters in the same row indicate significant differences (ANOVA, $P < 0.05$).

3.3. Chemical fractionation

The C, N and C/N values of the casts were significantly different to those of the control and non-ingested soils (Table 3). Both C and N values were higher. The non-ingested soil showed the lowest values.

The casts were significantly enriched in free organic matter, the least humified fraction. Although they were also richer in FFA, HA and non-extractable humin, and lower in FA and extractable humin than the control soil, these differences were not significant. Comparison of the results for the non-ingested and control soils shows a different pattern. The non-ingested soil was only richer in FA. All other fractions were less abundant, including a lower proportion of plant remains (free organic matter) and humins. In the control soil, the HA/FA ratio was approximately 1: this is normal for very impoverished soils like that of El Molar. However, the casts had ratios of greater than 1, indicating greater humification and a certain stability.

4. Discussion

The C and N contents of the soil at El Molar are very low, showing it to be very poor in nutrients. The casts deposited by *H. elisae* had higher C and N contents than either the control or the non-ingested soil, showing that organic material had been actively selected. This agrees with that known to occur in other earthworm species [7,33]. The C/N ratio decreased after the soil passed through the gut, which might be due to a greater proportional use of the C by the organism or also due to a small amount of N secretion. Together, this information strongly suggests that *H. elisae* searches out the richest organic components of the soil; these would be more nutritive and can be digested after breakdown and mixing during passage through the gut [24,28]. The lower C/N ratio implies a greater degree of humification [29]; the HA/FA ratio reflects the same and shows greater stability after its passage through the gut. *H. elisae* casts may therefore act as nutrient reservoirs in these poor soils. It is not surprising that plant roots frequently follow cast-filled earthworm galleries (field observation).

The granulometric analysis showed that when *H. elisae* is cultivated in 2 and 4 mm sieved soils, it ingests more clays and less coarse sands. This is similar to that seen in other earthworm species such as *Lumbricus terrestris* and *A. caliginosa* [27], *D. terraenigrae* and *M. anomala* [11] and *A. molleri* [30]. However, conflicting results have been obtained with species such as *M. anomala*, which ingests bulk soil without making any type of selection [20], and *P. corethrurus*, which in some experiments showed more coarse sands and less fine sands in its casts than in the control soil [19].

The differences between the composition of the *H. elisae* casts and the 4 mm control soil were not very great (82.59% total sands in control soil compared to 76.58% in casts, 11.89% clays in casts compared to 7.57% in control soil). This indicates that it makes only a weak selection of the soil it ingests. This selection varies, however, increases with the texture of the soil, e.g. in the < 2 μm mineral fraction, selection increased with increasing coarseness of texture. Such differences have also been reported by other authors who indicate that the sand content of casts is affected by the texture of the soil in which the earthworm lives [32].

However the action of soil sieving on aggregates is not considered here. Soil sieving can eliminate some silt and clay particles structured in aggregates modifying the real proportion of textural classes in sieved soil. On the other hand earthworm selection can act towards aggregates and free particles and not towards particles embedded in aggregates.

The physical fractionation (without destruction of the organic matter) and chemical fractionation results show that *H. elisae* ingests proportionally less of the coarse mineral fraction and more of the coarse organic fraction than is present in the soil; in other words it actively selects the least humified free organic matter (free organic matter and FFA, physical fraction = > 50 μm). Owing to the microflora in its gut, the earthworm therefore increases the decomposition and transformation of the most easily palatable organic matter. Though there were no significant differences with respect to the remaining organic fractions, the casts were very enriched in non-extractable humin. Also, the cast values for the 0–2 μm fraction show a certain consumption of the organic component of this size.

Martin (1991) and Martin et al. (1992a, b) found that *M. anomala* preferentially assimilate the organic fractions > 50 μm, accompanied by a few finer fractions (especially microbial metabolites and biomass). They state that, independent of their ecological category, earthworms feed mainly on recent soil organic pools and assimilate soil organic matter of the same age as do other decomposers. The present results suggest that *H. elisae* follows the same basic pattern.

5. Conclusion

In the experimental conditions of this paper *H. elisae* makes a weak selection of certain soil fractions. The 200–2000 μm mineral fraction is negatively selected, while the organic fraction of the same size (composed mainly of free organic matter) seems to be positively selected. This fraction would also seem to be partly digested and partly fragmented into smaller particles. The 0–2 μm fraction is significantly selected according to the granulometric analysis performed. The physical fractionation gave cast and non-ingested soil values suggesting that part of its organic component is digested. The high values for non-extractable humin in casts suggests that some of the organic components of this fraction (possibly microbial humins), might be used by the earthworm.

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