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Determination of the origin of the different growing abilities of two populations of *Millsonia anomala* (Omodeo and Vaillaud), a tropical geophageous earthworm

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Abstract

Individuals of the tropical endogeic earthworm *Millsonia anomala* from population of savannah patches cannot grow when introduced in a forest soil while forest individuals can grow in both soil types. Laboratory cultures in both soil types were performed to explain the differences observed. The quality of the soil organic matter or the available microorganisms present in the soil may be responsable of worms growth. The most likely hypothesis was that the ability to digest soil organic matter may be aquiered during the early stages of life when the gut is first colonized by particular soil microbial populations and that these microorganisms stay in the gut during the rest of the life-span.

Keywords: Earthworms, *Millsonia anomala*, intestinal micro-organisms, tropical, geophageous, digestion.

Détermination de l'origine des différents taux de croissance de deux populations de Millsonia anomala *(Omodeo et Vaillaud), un ver de terre géophage tropical.*

Résumé

Des individus de l'espèce de ver endogé géophage *Millsonia anomala* provenant d'une métapopulation de savanne ne peuvent pas grossir lorsqu'ils sont introduits dans un sol de forêt alors que les individus de forêt peuvent croître dans les deux milieux. Des élevages de vers en laboratoire dans les deux types de sol ont été menés pour expliquer les différences observées. La qualité de la matière organique ainsi que les microorganismes présents dans le sol peuvent être responsables du taux de croissance des vers. L'hypothèse la plus probable est que la capacité de digérer la matière organique du sol serait acquise dans les premiers moments de la vie quand pour la première fois des populations de microorganismes du sol envahissent le tube digestif du ver et que ces micro-organismes demeurent dans l'intestin du ver tout le reste de sa vie.

Mots-clés : Ver de terre, *Millsonia anomala*, micro-organismes intestinaux, tropical, géophage, digestion.

INTRODUCTION

Endogeic earthworms are a dominant component of communities in soils of humid tropical savannahs (Lavelle, 1983). Their high rates of ingestion and specific digestive processes allow them to grow in soils with very low organic matter contents.

Several authors assume that earthworms are able to produce enzymes to digest soil organic matter (Devigne and Jeuniaux, 1960; Parle, 1963; Urbasek and Pizl, 1991). But there is also strong evidence that digestion by some earthworms may be performed by micro-organisms in their gut (Lavelle *et al.*, 1983; Barois, 1987; Martin *et al.* 1987; Trigo and Lavelle, 1993). Worms produce assimilable compounds in their gut, which trigger high metabolic activity of certain bacteria present in the gut increasing soil organic matter degradation in the posterior part of

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the gut. For exemple several important enzymes as cellulase or mannanase which are found in the gut of the earthworm *Pontoscolex corethrurus* are actually produced by the ingested microflora and not the earthworm itself (Zhang *et al.*, 1993).

At Lamto (Côte d'Ivoire), the vegetation is a mosaic of grass and shrub savannahs and forest patches. Earthworm communities are dominated by *Millsonia anomala* (Omodeo and Vaillaud, 1967), a mesohumic endogeic Megascolecidae which comprises 50 to 80% of the overall worm biomass in the savannahs and forests (Lavelle, 1978). *M. anomala* was less abundant in the forest than in the savannah (respectively 32 $I.m^{-2}$ and 19 $I.m^{-2}$ sampling of december 1992).

Previous observations indicated that *M. anomala* taken from the savannah did not survive when introduced in forest soils whereas forest worms where not affected when grown in a savannah soil. The aim of this study was to characterize differences of the digestive abilities of the worms of the two origins (forest, savannah) and to determine the reasons for such differences.

The hypotheses tested in these experiments were:

1: in the forest ecosystem, decomposition of the litter and soil organic matter releases hydrosoluble organic compounds that are toxic to savannah earthworms;

2: micro-organisms of the forest soil are less efficient in assisting the worm in the degradation of soil organic matter than micro-organisms from the savannah; forest worms have become abble to grow with this less efficient microbes, while savannah worms have not;

3: in early life stages, eathworms can adapted to soil conditions, possibly through uptake of microbes, that stay in the gut during the rest of the life-span;

The experimental design included two parts, experiments with young worms grown in natural and sterilized soil to test hypotheses 1 and 2 and experiments with cocoons to test the third hypothesis. Responses of earthworms were evaluated by measuring soil ingestion rates, growth rates and growth efficiencies.

MATERIALS AND METHODS

The study was carried out at Lamto, Côte d'Ivoire (5°N,6°W) where the climate is tropical humid, with an average annual rainfall of 1200 mm. Vegetation would be a deciduous forest if the savannah was not maintained by regular burning. Vegetation of the forest plot was a *Celtus-Triplochiton* formation that has not been exploited for 15 years. The shrub savanna plot was dominated by two *Hyparrhenia* species (*Hyparenia smithiana* and *Hyparrhenia diplandra*). Shurbs were about 150 to 300 per hectare.

Table 1. – Main characteristics of the upper horizon (0-10 cm) at Lamto (Côte d'Ivoire).

	C (%)	N (%)	C/N	pH	Sand (%)	Silt (%)	Clay (%)
Savannah	0.85	0.59	14.4	5.9	78.0	17.0	6.0
Forest	1.18	1.20	9.8	6.8	85.0	10.5	4.5

Soils lie on a granitic bedrock and are classified as "sol ferrugineux tropicaux" (Riou, 1974) or Ferrasol (FAO). Soil samples were collected in the upper 10 cm of soil in a shrub savannah and in a plateau forest. They have a sandy texture and low C and N contents (*table* 1). C/N is higher in the savannah than in the forest in the upper 10 cm. pH is slightly acidic.

First experiment

Young *M. anomala* worms were collected from both soils in July 1992. The average fresh weight of each worm at the beginning of the experiment was 0.25 ± 0.02 g corresponding to the weight of 10 weeks old worms (Lavelle, 1978).

Worms were raised in laboratory conditions using the method of Lavelle (1975). The worms were cultured in soil which has been previously moistened (pF 2.5) and sieved through a 2-mm mesh. This technique separates casts produced by worms from non ingested soil.

Four treatments were imposed to assess growth rates of worms from the two sites in natural soils.

- 1: savannah worms in savannah soil;
- 2: forest worms in forest soil;
- 3: savannah worms in forest soil;
- 4: forest worms in savannah soil;

Three other treatments were also applied in order to test the hypothesis of the presence of compounds toxic for worms in the forest soil organic matter (SOM)(5) or the lower efficiency of forest soil microflora to assist the worm in the degradation of SOM.

- 5: savannah worms were grown in savannah soil added with a sterilised extract of the forest soil (treatment 5);
- 6: savannah worms were grown in sterilised savannah soil (24 hours at 105°C) inoculated with extracts of the savannah soil;
- 7: savannah worms were grown in sterilised savannah soil inoculated with extracts of the forest soil.

Extracts were obtained by adding 2 l of filtered water to 2 kg of fresh soil previously sieved at 2 mm, mixing mechanically for 2 hours and filtering with a 200 μm mesh size sieve. In treatment 5, the extract was sterilised for 2 hours at 100°C.

Culture media were prepared three days before the introduction of earthworms. Soil moisture content was

15% (pF=2.5); the dry matter added with the extract was respectively 0.13%, 0.12% and 0.11% of dry weight of soil in treatments 5, 6 and 7.

Four hundred g wet soil and 3 worms were put in each box. The experiments were carried out for 80 days with 4 replicates for each treatment; every ten days, the earthworms were weighed, the soil was changed and the casts were extracted from the culture medium and weighed.

Second experiment

Cocoons of *M. anomala* were collected in the grass savannah and plateau forest in November 1992. Cocoons found in the forest were incubated in forest soil; half of the cocoons found in the savannah were placed in forest soil and the second half in savannah soil.

Every three days the boxes containing the cocoons were checked and newly born worms were individually placed in boxes containing ca. 80g of soil moistened to field capacity. Worms were cultured in the soil in which they had been born. Every 7 days, worms were weighed and soil consumption was measured as in experiment 1.

Twenty three savannah worms were grown in the savannah soil and 20 in the forest soil. Eleven forest worms were grown in the forest soil.

After 4 to 5 weeks of individual culture, worms were placed in a larger box containing 350g of wet soil (three worms per box) and the culture was prolonged for 60 days with evaluations of each parameter every 10 days.

Three parameters were measured:

- Growth rate: $G = \frac{W_2 - W_1}{W_1}$ %.day^{-1}

W_1: fresh weight at time 1

W_2: fresh weight at time 2

- Ingestion rate

$$I = \frac{\text{ingested dry soil}}{(W_2 - W_1/2)} \text{ g.g fw.day}^{-1}$$

- Growth yield efficiency

$$Y = \frac{G}{\text{amount of C ingested}} \%.\text{mg}^{-1} \text{ C soil}$$

C ingested is calculated from soil C content and ingested soil

In experiment 1, the parameters were calculated for the 7 ten days periods; the mean of these seven values was used, for each replicate and treatment. A one-way anova was performed for each parameter.

RESULTS

Experiment 1

Growth of earthworms significantly differed in the four treatments (*table* 2).

In the savannah soil, worms from the two soils had similar growth rates, ingestion rates and growth yield efficiencies (*fig.* 1). Savannah worms were unable to grow in the forest soil unlike the forest worms, although they had similar ingestion rates. Furthermore the growth rate of forest worms was lower in the forest soil than in the savannah soil.

The addition of a non sterile forest extract to savannah soil to test the existence of a toxic compound did not affect the growth of savannah worms (treatment 5). Soil ingestion however was significantly lower. There was no evidence of the presence of toxic substances in the forest extract and the addition of this extract containing water-soluble organic matter induced a greater efficiency of earthworm growth (*fig.* 2).

The growth rate of savannah worms in sterile savannah soil added with non sterile forest or savannah soil extract (treatment 6 and 7) was not significantly different althrough it was lower than growth rate of the savannah worms in the savannah soil (treatment 1). The growth yield efficiency of the worms was higher with the savannah soil extract than with the forest soil extract.

Table 2. – Initial and final weight, growth rate, ingestion rate and growth yield efficiency of young *M. anomala* of different populations fed with natural and modified soils.
S W: savannah worms; S S: savannah soil; F W forest worms; F S: forest soil; FE: forest extract; SE: savannah extract; *: sterilised substrate.

	Initial weight (g)	Final weight (g)	Growth rate %.day^{-1}	Ingestion rate g soil.g^{-1}.day^{-1}	Yield efficiency %.dmg^{-1} C soil
1: S W / S S	0.25	0.56	1.65 a	20.5 a	7.3 a
2: S W / F S	0.25	0.22	0.00 b	21.0 a	2.2 a
3: F W / S S	0.20	0.44	1.50 a	24.5 a	5.8 a
4: F W / F S	0.22	0.35	0.60 c	18.2 a	5.1 a
1: S W / S S	0.25	0.56	1.65 a	20.5 a	7.3 a
>5: S W / S S+FE*	0.24	0.62	1.76 a	17.5 b	8.7 ac
6: S W / S S*+ FE	0.23	0.38	0.84 b	15.6 b	5.6 b
>7: S W / S S*+ SE	0.24	0.43	1.13 ab	15.0 b	9.2 c

Data from the same column with different letters are significantly different (p=0.05)

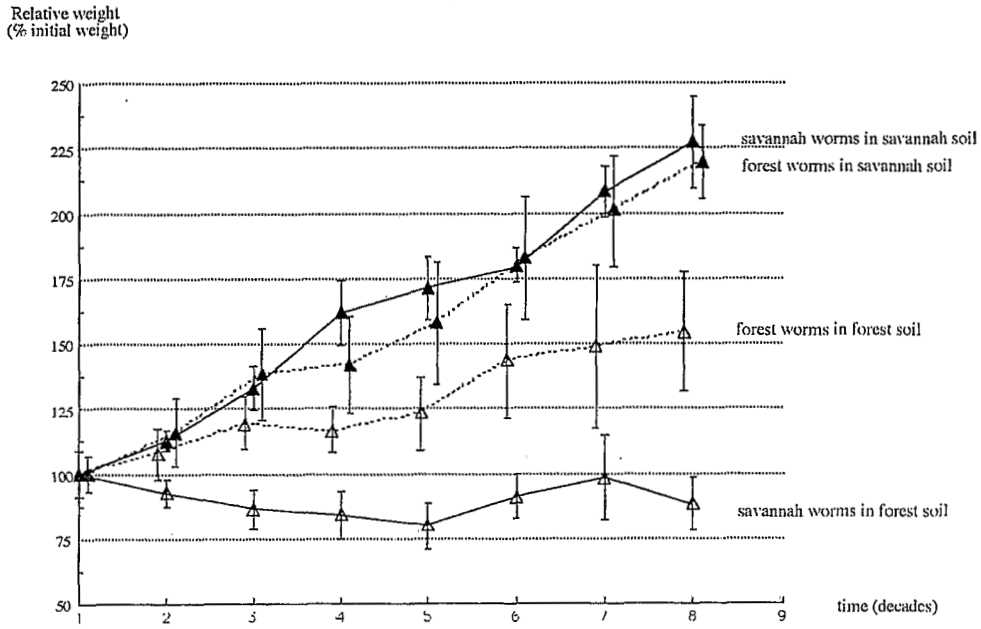

Figure 1. – Relative growth of the two worm types in forest and savannah soils (error = standard error) see abbreviations in *table* 2.

Experiment 2

Mean fresh biomass of newly hatched worms was 48±5 mg and there was no difference between forest and savannah individuals. At the end of the experiment the savannah worms grown in savannah soil weighed 0.47±0.3 g while the savannah and forest worms grown in the forest soil weighed 0.23±0.2 g and 0.23±0.3 g respectively. During the six first weeks of the experience, the growth rate of the worms in the two soil types was not very different. But after this period the growth rate of the savannah worms in the savannah soil increased whereas the growth rate of the worms in the savannah soil reminded constant (*fig.* 3).

The growth rate of the worms in the savannah soil was thus significantly higher than that of the worms in the forest soil. But neither the growth rate of the two worm types, nor their ingestion rates or growth yield

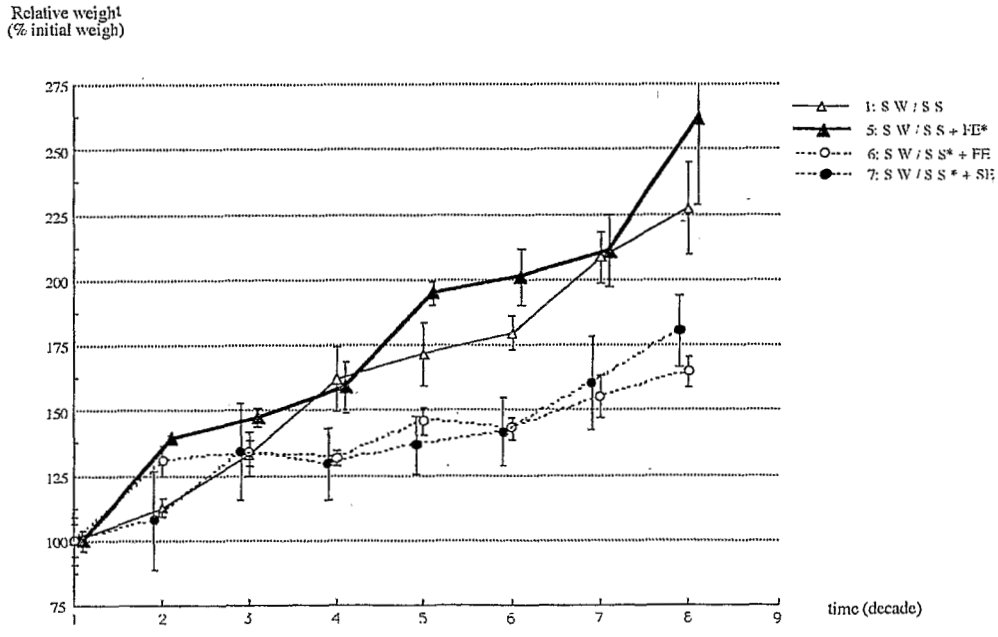

Figure 2. – Relative growth of savannah worms in different mediums derived from the savannah soil (error = standard error) see abbreviations in *table* 2.

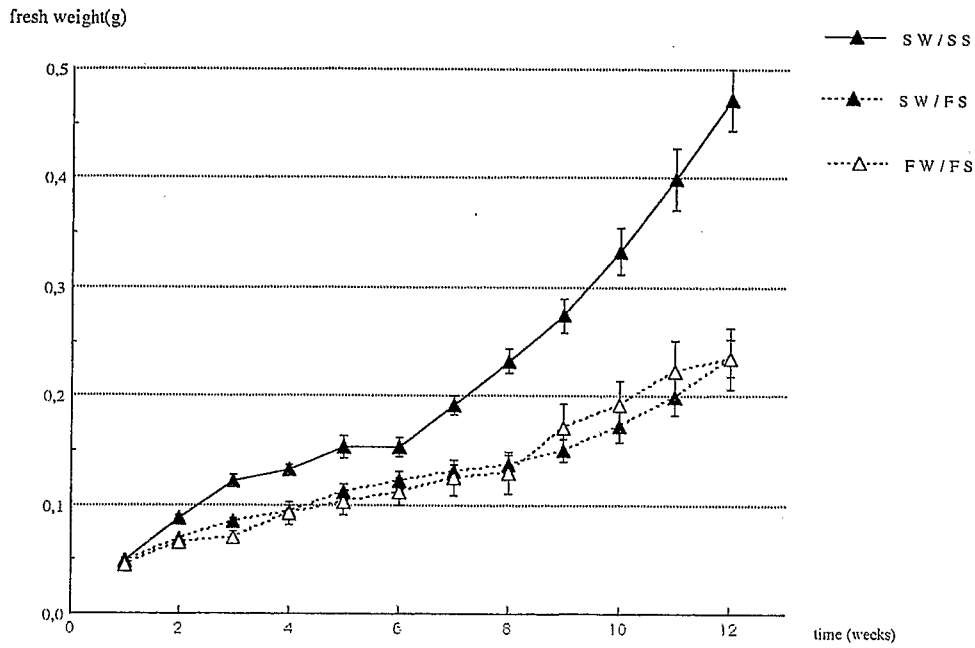

Figure 3. – Fresh weight of the two type of worms in forest and savannah soils (error = standard error) see abbreviations in *table* 2.

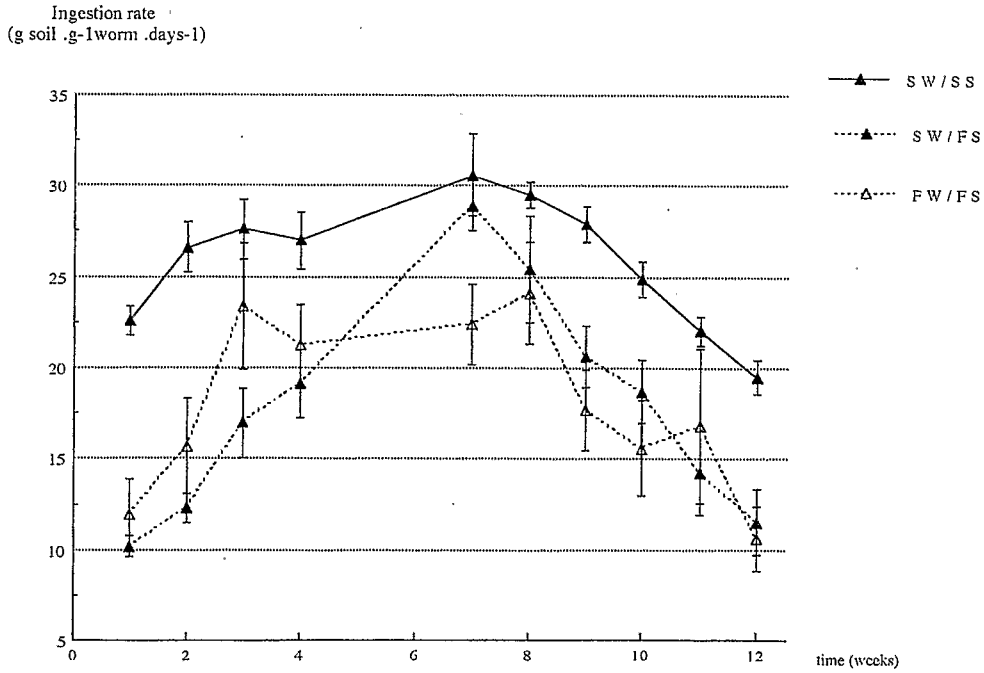

Figure 4. – Ingestion rate of the two types of worm in forest and savannah soils (error = standard error) see abbreviations in *table* 2.

efficiencies were different in the forest soil (*figs.* 3 and 4).

DISCUSSION

The forest soil is less favourable to the growth of *M. anomala* than savannah soil. Although earthworms borne in savannah have the same ingestion rate than in forest soils, they do not increase in weight. It is widely recognised that many temperate or tropical forests have much lower carrying capacities for earthworms than grasslands (e.g., review of Edwards and Lofty, 1977; Lavelle, 1983; Fragoso and Lavelle, 1992). Although reasons for such differences have rarely been directly addressed, drier soil conditions

in forests (Lavelle, 1983) and differences in quality and distribution patterns of organic inputs are likely to affect earthworm communities. In forests leaf litter appears to have higher content of polyphenolic compounds than grasses and decomposition is likely to release toxic compounds which may affect soil biota. To test this hypothesis, sterile watersoluble extracts of forest litter were added to a savannah soil with no significant effect on either growth, ingestion rate or growth yield efficiency. These results do not support the hypothesis of toxic compounds in the forest soil watersoluble extracts.

The other hypothesis tested considered the effect of differences in soil microflora between the two biomes. Numerous soil invertebrates are closely associated to microflora for their digestion processes (Kiffer and Benest, 1981; Ineson and Anderson, 1985; Breznak, 1984). A mutualist digestion model has been proposed in which the earthworm creates suitable conditions for microbial populations in its gut by adding water and intestinal mucus, a easily assimilable source of organic matter (Barois, 1987; Martin *et al.*, 1992). After a phase of strong activation in the foregut, in which they use first mucus as a food resource, microorganisms then become able to digest SOM in the posterior part of the gut releasing assimilable compounds for their own benefit and that of the worm. Measurement of microbiological activities, extraction and analyses of water soluble compounds in different parts of the gut and enzyme analyses support this hypothesis.

In our study, it was assumed that microorganisms from forest soils might be less efficient than savannah ones in assimilating SOM in earthworm guts. Significant differences of growth yield efficiency between treatment 6 (sterilised savannah soil + non sterile savannah extract) and treatment 7 (sterilised savannah soil + non sterile forest extract) seem to support this hypothesis; although the lack of a treatment associating a non sterile savannah soil to sterile savannah extract to test the energetic value of extracts does not allow to conclude at that stage. The yield efficiencies obtained in this experience were lower to those of the experience of Martin and Lavelle (1992) conducted with the same worm specie. But the worms were fed on soil supplemented with fresh plant material which appeared to be good energetic ressources for *M. anomala.*

There was no difference in soil ingestion and growth rates of newly hatched earthwoms from the forest and savannah. This result indicates that the ability to digest forest SOM may be probably acquired during the early stages of life when the gut is first colonized by soil microbial populations. Microflora associated with the worm gut for digestion of SOM can be more highly linked to the gut than was previously considered. A microbiological and a biochemical approach to caracterised the soil microflora and the soil humus would be necessary to test with certainty whether the different growing abilities are exclusively linked to the microorganisms.

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