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Is the aestivation of the earthworm *Hormogaster elisae* a paradiapause?

Darío J. Díaz Cosín,[a] María Pilar Ruiz, Marta Ramajo, and Mónica Gutiérrez

Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain

Abstract. We report the results of a laboratory study of the aestivation of *Hormogaster elisae* to determine the nature of the inactive period (diapause or quiescence) and to evaluate the influence of soil moisture, temperature, season, and earthworm body weight on the process. The results showed that specimens of *H. elisae* underwent facultative diapause—paradiapause—characterized by the construction of aestivation chambers in which the animal coils up and its activity decreases. Soil moisture appeared to be the most important environmental factor involved in the onset of aestivation. Temperature and time of year also had some influence, but earthworm body weight appeared to have none. Aestivating earthworms showed a decrease of 41.6% in mean body weight. Once replaced in soil with 20% moisture content, they took 6.4 ± 3.1 d to exit their chambers and another 6.5 ± 3.6 d to recover their initial body weight.

Additional key words: earthworms, aestivation chambers, diapause, quiescence

When environmental conditions are too severe for normal activity to continue, earthworms can react in different ways. For example, when soil moisture content is inadequate, they can move vertically or horizontally, form cocoons, or modify their metabolism to reduce water loss, entering into what is known as a "resting state," "dormant stage," or "state of inactivity" (Lee 1985; Edwards & Bohlen 1996). This reduction in metabolic activity has been described in the literature as either diapause (obligatory or facultative) or quiescence (Satchell 1967; Nordström & Rundgren 1974). It has been suggested that obligatory diapause might be controlled by neurosecretions (Lee 1985). The process begins at a certain time of the year, and once underway its duration is independent of artificial manipulation of environmental factors. The earthworms stop feeding, empty their gut, and construct an aestivation chamber in which they remain coiled up in a state of low activity (Michon 1957; Saussey 1966). Facultative diapause, or paradiapause (Saussey 1966), is different from the obligatory type in that it may terminate at any time when environmental conditions become suitable for normal activities.

Quiescence is defined as a direct response to variation in an environmental factor such as soil moisture or temperature; this response ends as soon as conditions become favorable. In quiescence, earthworms stop feeding and remain in a torpid state, but they do not make special chambers. Bouché (1972) distinguished three types of quiescence: anhydrobiosis, reaction to toxicity, and hibernation. The latter is initiated when temperatures fall below 2°–4°C. The worms coil up in small soil spaces just below the superficial frozen soil layer. Lee (1985) indicates that the differences between obligatory and facultative diapause and quiescence can be site specific and that a gradation exists between these processes with variations in response to the severity of the stress suffered by individual populations. Variations may be seen in the response of a species with respect to the time of year; e.g., *Scherotheca gigas rodana* var. *gallisiani* Bouche 1972, an endogeic Mediterranean species, undergoes diapause in summer and quiescence in winter (Gallisian 1975).

As the majority of the available aestivation data come from field observations of lumbricids, of interest is a better understanding of the processes of inactivity in other families of terrestrial oligochaetes. We studied a Mediterranean species, *Hormogaster elisae* Álvarez 1977 (Hormogastridae), an endogeic species endemic to the center of the Iberian Peninsula (Madrid and Segovia), found in soils poor in organic matter and whose habitat is subject to large variations in soil temperature and moisture over the year (strong summer drought). In response to moisture

[a] Author for correspondence.
E-mail: dadico@bio.ucm.es

content, this species moves vertically and builds aestivation chambers as deep as 50 cm in the soil (Valle et al. 1997, 1999); e.g., we found aestivation chambers during a drought in May 2003 at El Molar. Our aim was to determine, by means of laboratory experiments, the nature of the inactive period in this species and to evaluate the influence of soil moisture, temperature, season, and earthworm body weight on the process.

Methods

Soils and earthworms were collected during April and October 2000, and April 2001 and 2002 at El Molar (Madrid), UTM 30TVL5210, by manual excavation and hand sorting. The worms were kept in the laboratory in the same soil (El Molar) until they were used for the experiments. Details of the soil, climate, and vegetation characteristics of El Molar can be found in Garvín et al. (2000).

Seasonal cultivation

The earthworms were separated into three weight classes: small (<1 g), medium (1–2 g), and large (>2 g) (this distribution was not an effort to describe different life stages in the earthworms). Three different soil moistures (10%, 15%, and 20% water content on a dry mass basis) and temperature treatments (growth chamber, laboratory temperature, and external temperature) were combined for a total of nine treatments. Each treatment was replicated 3×, one for each weight class (27 microcosms in total).

Growth chamber temperature was maintained at 18°C. Laboratory temperatures during the study period (averages of maximal and minimal) were 25.64°C and 22.15°C in spring, 29.2°C and 25.47°C in summer, 22.82°C and 19.3°C in autumn, and 22.68°C and 19.3°C in winter. The averages of maximal and minimal external temperatures, which fluctuated between day and night, were 28.3°C and 7.1°C in spring, 36.4°C and 14.3°C in summer, 17.9°C and 12.5°C in autumn, and 11.8°C and 4.6°C in winter (Madrid, University City Weather Station).

The microcosms consisted of plastic boxes containing 2-mm sieved soil, from the collection site, adjusted to the desired moisture. Three earthworms of the same weight class were introduced into each microcosm (81 earthworms in total). The quantity of dry soil used (400 g for small earthworm microcosms and 650 g for medium and large ones) was adequate to prevent food from becoming a limiting factor to activity. This quantity was calculated from the mean cast production rate for this species (3.34 g dry casts g^{-1} live earthworm mass per day; Díaz Cosín et al. 1996). The microcosms at external temperature were put on a window ledge, shaded by an outer lattice window.

The duration of the experiment was 2 weeks. Moisture content was maintained by measuring the weight change of the microcosms, and adding water as necessary every 2–3 d. At the end of the experiment, the microcosms were examined, and the number of aestivating (pale, coiled into a tight ball in a mucus-lined chamber) and dead worms was counted. The same procedure was repeated in May 2000 (spring), June–July 2000 (summer), October–November 2000 (autumn), and February 2001 (winter).

The chi-square of the G-test (calculated previously using contingency tables) and log-linear analysis (employing Statistica for Windows 4.5, Statsoft, Tulsa, OK) were used to analyze the influence of soil moisture, temperature treatment, season, and earthworm weight class on the number of aestivating earthworms per microcosm. The G-test is appropriate to test the combined influence of categorical variables. The log-linear analysis was used to search for a "good model" that includes the least number of interactions necessary to fit the data. Temperature and season are interdependent variables; therefore, only temperature was used for the statistical analysis, or season using the data from the constant temperature treatment (18°C, growth chamber) to detect whether there is an "internal clock" that regulates the aestivation, independent of temperature variations.

Additional experiments

The results obtained suggested that further experiments might be undertaken to investigate the process more fully. The influence of temperature was complemented by a second series of experiments with two new temperatures (13°C and 23°C) in a culture chamber. In May 2002, three different soil moistures (10%, 15%, and 20% water content on a dry mass basis) and two temperatures (13°C and 23°C in growth chambers) were combined for a total of six treatments, with three replicates of each treatment (18 microcosms in total). Each microcosm consisted of a plastic box containing 650 g of air-dried soil, sieved at 2 mm and adjusted to the desired moisture content. Three earthworms, without separation in weight class (or of different size), were introduced into each microcosm (54 earthworms in total).

The duration of the experiment was 2 weeks and moisture content was maintained by adding water as necessary every 2–3 d. At the end of the experiment, the microcosms were examined and the number of

aestivating worms was counted. The G-test (employing Statistica for Windows 4.5) was used to analyze the combined influence of soil moisture and temperature on the number of aestivating earthworms per microcosm.

In June–July 2002, a third series of experiments was carried out to determine the existence of a soil moisture content below which the earthworms aestivate independent of temperature. The microcosms were plastic boxes containing 200 g of air-dried soil, sieved at 2 mm, taken to 5% moisture, and cultivated at 13°C, 18°C, and 23°C in growth chambers, the same temperatures used in the previous experiments. Six microcosms, each with one earthworm, for each temperature were used (18 microcosms in total). The duration of the experiment was 2 weeks and moisture content was maintained as described above, after which the microcosms were examined and the number of aestivating worms was counted.

Aestivation chambers

To induce the building of aestivation chambers, 27 microcosms were carried out. These were plastic boxes of 0.5 L capacity, containing 200 g of air-dried soil, sieved at 2 mm, taken to 10% moisture, and cultivated at external temperature as described above. Earthworms were weighed and one was placed in each microcosm. The duration of the experiment was 4 months (May–September 2001). The moisture content was maintained constant by adding water as necessary every 7–10 d.

At the end of the experiment, the microcosms were examined and the aestivation chambers were carefully separated from the soil, cleaned, measured, and weighed. Each aestivation chamber was then placed in a microcosm to observe the time needed, in optimal moisture conditions (20% moisture content), for the earthworm to return to normal activity, marked by exit from the aestivation chamber. These microcosms were cultivated at external temperature. The aestivation chambers were examined daily and the weight of each earthworm was recorded as it came out of its chamber. Each earthworm was weighed daily to determine the time required for the worm to return to its initial weight.

Results

Seasonal cultivation

The largest percentages of aestivating earthworms occurred in microcosms with 10% soil moisture

Table 1. Number (and percentage) of aestivating individuals of *Hormogaster elisae* under experimental variables; n = number of earthworms per experiment.

Moisture (%)	Temperature	Spring ($n = 79$)	Summer ($n = 80$)	Autumn ($n = 81$)	Winter ($n = 81$)	Total
10	18°C	7 (77.78) $n = 9$	8 (88.89) $n = 9$	3 (33.33) $n = 9$	4 (44.44) $n = 9$	22 (61.11) $n = 36$
	Laboratory	9 (100) $n = 9$	9 (100) $n = 9$	2 (22.22) $n = 9$	4 (44.44) $n = 9$	24 (66.67) $n = 36$
	External	2 (22.22) $n = 9$	7 (77.78) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	9 (25) $n = 36$
15	18°C	1 (11.11) $n = 9$	3 (33.33) $n = 9$	0 (0) $n = 9$	1 (11.11) $n = 9$	5 (13.89) $n = 36$
	Laboratory	4 (44.44) $n = 9$	6 (66.67) $n = 9$	1 (11.11) $n = 9$	0 (0) $n = 9$	11 (30.56) $n = 36$
	External	1 (11.11) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	1 (2.78) $n = 36$
20	18°C	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 36$
	Laboratory	0 (0) $n = 8$	1 (12.5) $n = 8$	0 (0) $n = 9$	0 (0) $n = 9$	1 (2.94) $n = 34$
	External	0 (0) $n = 8$	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 9$	0 (0) $n = 35$
Total		24 (30.38)	34 (42.5)	6 (7.41)	9 (11.11)	73 (22.74)

(Table 1). All earthworms at laboratory temperature aestivated in spring and summer at this moisture content. However, these were the only two cases with 100% aestivation of earthworms, suggesting that the soil moisture, although a very important factor, did not by itself induce aestivation of all earthworms, in the interval used in this experiment (10%, 15%, and 20%).

The percentage of aestivating worms across all moisture contents was higher at laboratory temperature (34%) than at 18°C (25%) and external temperature (9.4%) (Table 1). At 10% soil moisture content, temperature exerted a greater influence than at 15% and 20% soil moisture; e.g., in spring, 100% of the earthworms aestivated at laboratory temperature, 77.8% at 18°C, and only 22.2% at external temperature, whereas at 20% soil moisture no earthworms aestivated, regardless of temperature. The interaction between soil moisture, temperature, and earthworm body weight had no significant influence on the number of aestivating earthworms per microcosm for the total year (G-test, $p = 0.4376$). Therefore, earthworm body weight had no influence on the number of aestivating earthworms per microcosm and, therefore, the results are shown without separation into weight classes in Table 1.

The interaction between soil moisture and temperature had a significant influence (G-test, $p = 0.0002$). However, within each season, the interaction between soil moisture and temperature did not significantly affect the number of aestivating earthworms per microcosm (G-test, $p = 0.8948$ in spring, $p = 0.1290$ in summer, $p = 0.8110$ in autumn, and $p = 0.8656$ in winter). To check these results, a log-linear analysis was conducted to detect which variables (soil moisture, temperature, or earthworm body weight) best explain the distribution of the number of aestivating earthworms per microcosm. The best model, with the least number of interactions, was that which included only soil moisture (99.99% of fit).

Seasonality was well defined, the highest percentages of aestivation were in summer (40.7%) and spring (29.6%), while very few worms aestivated in winter (18.5%) and autumn (11.1%) per microcosm cultivated at 18°C. The interaction of soil moisture and season had significant effects on the observed number of aestivating earthworms (G-test, $p = 0.0179$).

Additional experiments

Here, the greatest percentage of aestivating worms was obtained at 10% soil moisture and 23°C (Table 2), i.e., the number of aestivating earthworms increased with decreasing soil moisture and increasing temperature. However, the interaction of soil moisture and temperature had no significant influence on the number of aestivating earthworms per microcosm (G-test, $p = 0.5112$). In the experiment conducted with microcosms at 5% soil moisture, 66.7% of the earthworms aestivated at 13°C and 100% at 18°C and 23°C.

Table 2. Number (and percentage) of aestivating individuals of *Hormogaster elisae* in the additional experiments (cultures in growth chambers; second series, three moistures, two temperatures, nine individuals per treatment; third series, 5% moisture, three temperatures, six individuals per treatment).

Moisture (%)	Temperature (°C)			
	13	18	23	Total
5	4 (66.66)	6 (100)	6 (100)	16 (88.88)
10	1 (11.11)	—	6 (66.66)	7 (38.88)
15	0 (0)	—	1 (11.11)	1
20	0 (0)	—	0 (0)	0

Aestivation chambers

All 27 specimens in this experiment made aestivation chambers after 4 months of cultivation at 10% moisture. The earthworms lay coiled up inside the chamber, partially dehydrated (Fig. 1A). The chambers were brown, ellipsoid in shape, with a mean size of $23.9 \pm 3.3 \times 21.6 \pm 3.1 \times 19 \pm 3.2$ mm and a weight of 6.35 ± 2.83 g (Fig. 1B). The internal surface was more or less smooth and covered with mucus; sometimes it was possible to see the mold of the pygidium and the last segments in the casts that formed the chamber's wall (Fig. 1C). The external wall was formed of a very compact mixture of soil and casts.

The initial average body weight of earthworms was 3.5 ± 0.7 g and the loss of weight over the experimental period was 41.6%. In soil at 20% moisture, the animals returned to activity within an average of $6.4 \pm 3.1\ d^{-1}$, leaving their chambers by perforating a small hole in the wall. Once normal activity was restored, the worms took an average of 6.5 ± 3.6 d to return to their initial weight.

Discussion

Soil moisture is an important factor in the onset of the aestivation process in *Hormogaster elisae,* as also observed in other species. The importance of soil

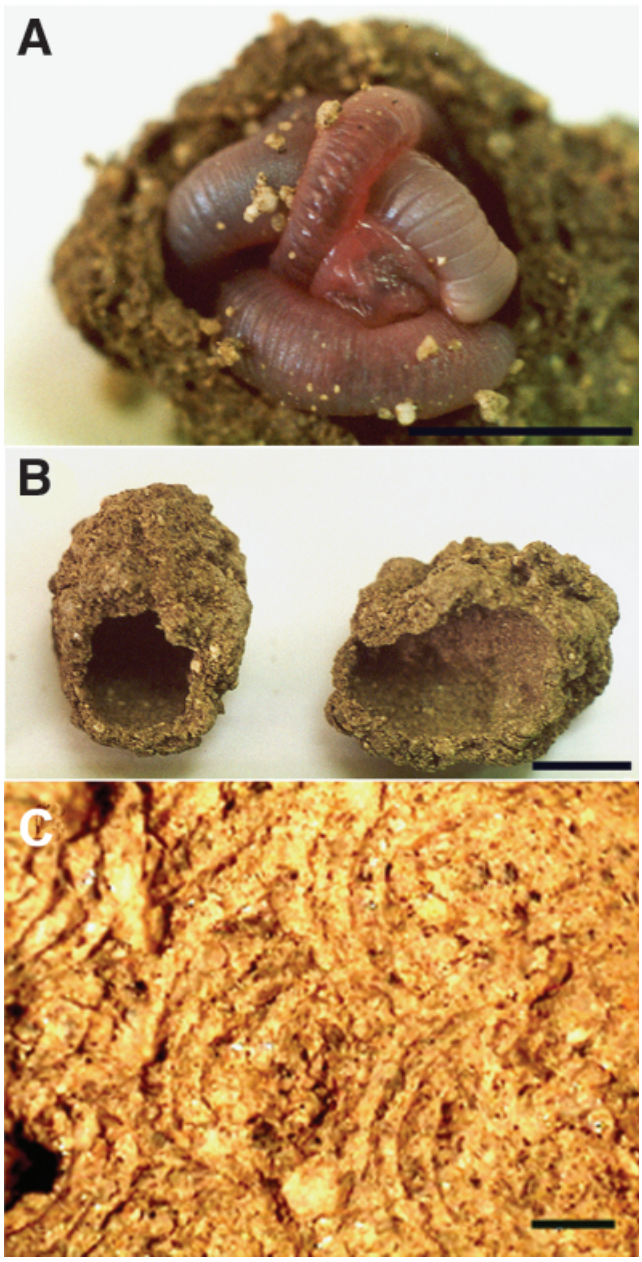

Fig. 1. Aestivation chambers in *Hormogaster elisae*. **A.** Individual coiled inside its aestivation chamber. **B.** Two aestivation chambers partially opened. **C.** Detail of the inner surface of an aestivation chamber. Note the molds made by the posterior body segments while compacting the casts during the chamber-building process. Scale bar, A, B, 1 cm; C, 1 mm.

moisture might be due to the fact that earthworms, whose water content in laboratory cultures is 65–75% of their total body weight (Oglesby 1969), are obliged to lose body water in large amounts to keep the body surface moist (cutaneous respiratory system) and through hypotonic urine (Lee 1985). A fall in soil moisture content below certain values prevents recovery from water loss; therefore, slowing metabolism and entering aestivation is one option to prevent the water loss. In our experiments, we did not record what proportion of the earthworm weight reduction was due to water or tissue loss, but the rapid recovery to initial weight (in $\sim$1 week) would suggest that the major part is simply water loss.

At 20% soil moisture, this species remains active in the laboratory (only one aestivated); even 15% soil moisture seems sufficient to meet most of their water requirements (17 earthworms aestivated). At 10% and 5% soil moisture, the percentage of aestivating worms rises, although these soil moisture contents were not enough so that, after 2 weeks, all of the earthworms had aestivated; however, 10% soil moisture over 4 months promoted the aestivation of all of the earthworms. This suggests that not only is the soil moisture content important but also the duration of low soil moisture content.

Temperature influences the activity, metabolism, growth, respiration, and reproduction of earthworms (Edwards & Bohlen 1996). Usually, temperature is not a conclusive factor, but becomes important for aestivation in *H. elisae* under certain soil moisture conditions. Earthworms have a limited capacity for cooling themselves by evaporating water from their body surface, but this is only effective when water contents are potentially high and the risk of desiccation is low (Hogben & Kirk 1944).

Adults and juveniles of *H. elisae* showed no difference in their aestivation strategies, which does not agree with that reported by Jiménez et al. (1998) for *Martiodrilus carimaguensis* Jimenez & Moreno in press (Oligochaeta, Glossoscolecidae), an anecic species in which adults are active for 8 months year^{-1} but in which juveniles enter diapause 3–4 months earlier than adults.

Our results suggest that in *H. elisae* there is no diapause in the strict sense as, when soil moisture is high (20%), very few of the worms aestivated. This clearly indicates that there is no "internal clock" independent of environmental factors. It is therefore a different type of process—paradiapause, or quiescence—that is more dependent on environmental factors than internal factors. Although soil moisture is the most important factor, temperature and season exert some influence on the aestivation of this species. The presence of aestivation chambers signals a paradiapause-type process, as these structures have not been described in earthworms that undergo quiescence.

Quiescence is an adaptation of endogeic species (Bouché 1977; Saussey 1981) such as *Allolobophora rosea* Savigny 1826 (Nordström 1975), *A. caliginosa*

SAVIGNY 1826 (Gerard 1967), *Octolasion lacteum* ORLY 1881 (Avel 1959), and *Millsonia anomala* OMODEO 1954 (Lavelle 1971). Diapause can be facultative (''paradiapause'' according to Saussey 1966) or obligatory, and is typical of anecic and epigeic species (Bouché 1977; Saussey 1981) such as *Lumbricus festivus* SAVIGNY 1826 (Oglesby 1969) and *A. longa* (Nordström 1975). There are exceptions; individuals of *Eisenia fetida* are typically epigeic and undergo quiescence (Avel 1959). It might be expected that *H. elisae,* typically endogeic, undergoes quiescence; but however, does not agree with our results, which indicate that this species undergoes paradiapause.

The aestivation chambers formed by individuals of *H. elisae* are similar to those described by Jiménez et al. (2000) for *Acanthodrilinae* sp., Glossoscolecidae Gen. nov. 1, and *M. heterostichon* in Mexico and Colombia. In *H. elisae,* earthworms respond directly to environmental change, although their response time may vary (6.4 ± 3.1 d) after moistening the soil. There are no data on other species to allow a comparison, or on any of the factors that regulate this process.

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